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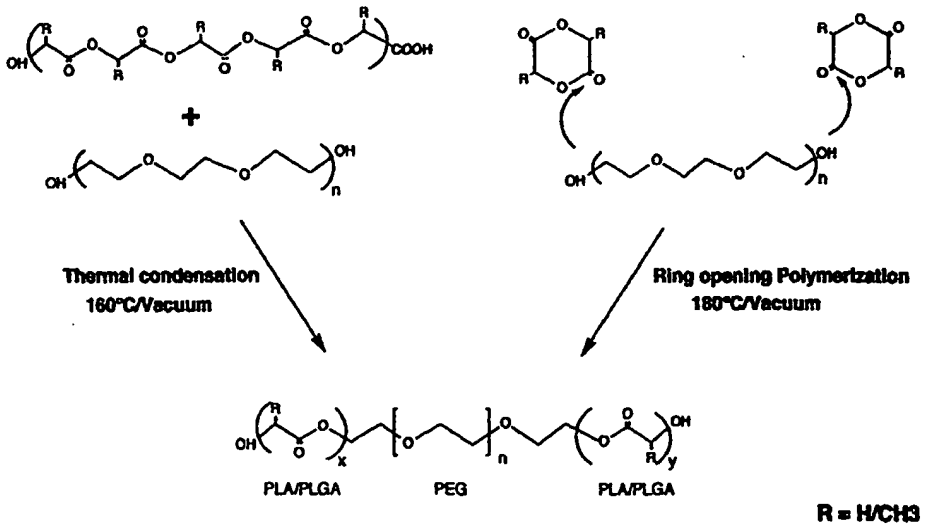

(A-B-A) BLOCK COPOLYMER

(57) Abstract

The present invention relates generally to the development of pharmaceutical compositions which provide for sustained release of biologically active polypeptides. More specifically, the invention relates to the use of thermosensitive, biodegradable hydrogels, consisting of a block copolymer of poly(d, l– or l–lactic acid) (PLA) or poly(lactide–co–glycolide) (PLGA) and polyethylene glycol (PEG), for the sustained delivery of biologically active agents.

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THERMOSENSITIVE BIODEGRADABLE HYDROGELS FOR SUSTAINED DELIVERY OF BIOLOGICALLY ACTIVE AGENTS

FIELD OF THE INVENTION

The present invention relates to the use of thermosensitive, biodegradable hydrogels, consisting of a block copolymer of poly(d,l- or l-lactic acid)(PLA) or poly(lactide-co-glycolide)(PLGA) and polyethylene glycol (PEG), for the sustained delivery of biologically active agents.

BACKGROUND OF THE INVENTION

Due to recent advances in genetic and cell engineering technologies, proteins known to exhibit various pharmacological actions *in vivo* are capable of production in large amounts for pharmaceutical applications. Such proteins include erythropoietin (EPO), granulocyte colony-stimulating factor (G-CSF), interferons (alpha, beta, gamma, consensus), tumor necrosis factor binding protein (TNFbp), interleukin-1 receptor antagonist (IL-1ra), brain-derived neurotrophic factor (BDNF), kerantinocyte growth factor (KGF), stem cell factor (SCF), megakaryocyte growth differentiation factor (MGDF), osteoprotegerin (OPG), glial cell line derived neurotrophic factor (GDNF) and obesity protein (OB protein). OB protein may also be referred to herein as leptin.

Because proteins such as leptin generally have short *in vivo* half-lives and negligible oral bioavailability, they are typically administered by frequent injection, thus posing a significant physical burden on the patient (e.g., injection site reactions are particularly problematic with many leptin

formulations) and associated administrative costs. As such, there is currently a great deal of interest in developing and evaluating sustained-release formulations. Effective sustained-release formulations can provide a means of controlling blood levels of the active ingredient, and also provide greater efficacy, safety, patient convenience and patient compliance. Unfortunately, the instability of most proteins (e.g. denaturation and loss of bioactivity upon exposure to heat, organic solvents, etc.) has greatly limited the development and evaluation of sustained-release formulations.

Biodegradable polymer matrices have thus been evaluated as sustained-release delivery systems. Attempts to develop sustained-release formulations have included the use of a variety of biodegradable and non-biodegradable polymer (e.g. poly(lactide-co-glycolide)) microparticles containing the active ingredient (see e.g., Wise et al., *Contraception*, 8:227-234 (1973); and Hutchinson et al., *Biochem. Soc. Trans.*, 13:520-523 (1985)), and a variety of techniques are known by which active agents, e.g. proteins, can be incorporated into polymeric microspheres (see e.g., U.S. Patent No. 4,675,189 and references cited therein).

Utilization of the inherent biodegradability of these materials to control the release of the active agent and provide a more consistent sustained level of medication provides improvements in the sustained release of active agents. Unfortunately, some of the sustained release devices utilizing microparticles still suffer from such things as: active agent aggregation formation; high initial bursts of active agent with minimal release thereafter; and incomplete release of active agent.

Other drug-loaded polymeric devices have also been investigated for long term, therapeutic treatment of various diseases, again with much attention being directed to polymers derived from alpha hydroxycarboxylic acids, especially lactic acid in both its racemic and optically active form, and glycolic acid, and copolymers thereof. These polymers are commercially available and have been utilized in FDA-approved systems, e.g., the Lupron Depot™, which consists of injectable microcapsules which release leuprolide acetate for about 30 days for the treatment of prostate cancer.

Various problems identified with the use of such polymers include: inability of certain macromolecules to diffuse out through the matrix; deterioration and decomposition of the drug (e.g., denaturation caused by the use of organic solvents); irritation to the organism (e.g. side effects due to use of organic solvents); low biodegradability (such as that which occurs with polycondensation of a polymer with a multifunctional alcohol or multifunctional carboxylic acid, i.e., ointments); and slow rates of degradation.

The use of polymers which exhibit reverse thermal gelation have also been reported. For example, Okada et al., Japanese Patent Application 2-78629 (1990) describe biodegradable block copolymers synthesized by transesterification of poly(lactic acid)(PLA) or poly(lactic acid)/glycolic acid (PLA/GA) and poly(ethylene glycol)(PEG). PEGs with molecular weights ranging from 200 to 2000, and PLA/GA with molecular weights ranging from 400 to 5000 were utilized. The resultant product was miscible with water and formed a hydrogel. The Okada et al.

reference fails to provide any demonstration of sustained delivery of drugs using the hydrogels.

Cha et al., U.S. Patent No. 5,702,717 (Dec. 30, 1997) describe systems for parenteral delivery of a drug comprising an injectable biodegradable block copolymeric drug delivery liquid having reverse thermal gelation properties, i.e., ability to form semi-solid gel, emulsions or suspension at certain temperatures. Specifically, these thermosensitive gels exist as a mobile viscous liquid at low temperatures, but form a rigid semisolid gel at higher temperatures. Thus, it is possible to use these polymers to design a formulation which is liquid at room temperature or at lower temperature and below, but gels once injected, thus producing a depot of drug at the injection site. The systems described by Cha et al. utilize a hydrophobic A polymer block comprising a member selected from the group consisting of poly(α-hydroxy acids) and poly(ethylene carbonates) and a hydrophilic B polymer block comprising a PEG. The Cha et al. system requires that less than 50% by weight hydrophobic A polymer block be utilized and greater than 50% by weight hydrophilic B polymer block be utilized. Interestingly, however, it appears that several of the disclosed hydrogels might not be commercially useful in that the lower critical solution temperature (LCST) for many of the gels is greater than 37°C. Although Cha et al. propose use of their hydrogels for controlled release of drugs, no such demonstration is provided.

Martini et al., *J. Chem. Soc.*, 90(13):1961-1966 (1994) describe low molecular weight ABA type triblock copolymers which utilize hydrophobic poly(ε-caprolactone)(PCL) and PEG. Unfortunately, *in*

vitro degradation rates for these copolymers was very slow, thus calling into question their ability as sustained-release systems.

Stratton et al., PCT/US97/13479 (WO 98/02142) January 22, 1998, describe pharmaceutical compositions comprising a polymeric matrix having thermal gelation properties, for the delivery of proteins. The class of block copolymers described are generically referred to as polyoxyethylene-polyoxypropylene condensates (also known as Pluronics). Unfortunately, systems utilizing Pluronics suffer from the fact that they are toxic to body organs and are nonbiodegradable. Moreover, only high molecular weight Pluronics at higher concentrations (25-40 wt.%) exhibit thermoreversible gelation.

It is thus the object of the present invention to provide thermosensitive, biodegradable hydrogels for the sustained delivery of drugs. The hydrogels of the present invention utilize copolymer compositions which provide for instant gelation, and which possess the necessary rate of degradation to make use of the hydrogels commercially practical.

SUMMARY OF THE INVENTION

In one embodiment, the present invention provides pharmaceutical compositions comprising an effective amount of a biologically active agent incorporated into a polymeric matrix, said polymeric matrix comprising a block copolymer which is biodegradable, exhibits thermal gelation behavior, and is capable of providing for the sustained-release of the biologically active agent.

In another embodiment, the present invention provides a method for the parenteral administration of

a biologically active agent in a biodegradable polymeric matrix to a warm blooded animal, wherein a gel depot is formed within the body of said animal and the biologically active agent is released from the depot at a controlled rate concomitant with biodegradation of the polymeric matrix.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the two methods by which the A-B-A block copolymers of the present invention can be prepared.

Figure 2 depicts the *in vitro* release characteristics of leptin released from a hydrogel (PLGA/PEG (74%/26% w/w)). % protein released is plotted vs. time (days).

Figure 3 is a photograph of an SDS-PAGE gel characterizing samples of leptin released from a hydrogel on various days. Lane 1 is a leptin standard; Lane 2 and 15 contain molecular weight markers; and Lanes 3-14 represent leptin samples at day 1-12, respectively.

Figure 4 depicts the *in vivo* bioactivity for various leptin-containing hydrogel (PLGA/PEG (74%/26% w/w)) formulations. The -✱- depicts a 20mM acetate, pH 4.8, buffer control, 100µl on day 0; -●- depicts a hydrogel (74%/26%) control, 100µl on day 0; -◆- depicts leptin (20 mg/mL), 100 mg/kg, 100µl on day 0; -Δ- depicts leptin (2 mg/mL), 10 mg/kg, 100µl daily; -▲- depicts a leptin-containing hydrogel (PLGA/PEG (74%/26% w/w)), 20 mg/mL leptin, 100 mg/kg, 100µl on

day 0; and -■- depicts a leptin-containing hydrogel (PLGA/PEG (74%/26% w/w)), 20 mg/mL leptin, 200 mg/kg, 200µl on day 0. % body weight change (from the day 0 body weight) is plotted vs. time (days).

Figure 5 depicts the pharmacokinetics for a leptin-containing hydrogel (PLGA/PEG (74%/26% w/w)) (-▲-) and leptin solution (-◆-). Serum leptin concentration (ng/mL) is plotted vs. time (hours).

Figure 6 depicts the *in vitro* release characteristics for GCSF from a GCSF-containing hydrogel (PLGA/PEG (74%/26% w/w)) (-◆-) and Fc-OPG from a Fc-OPG-containing hydrogel (PLGA/PEG (74%/26% w/w)) (-■-). % protein released is plotted vs. time (days).

Figure 7 is a photograph of an SDS-PAGE gel characterizing samples of GCSF released from a hydrogel on various days. Lane 1 is a leptin standard; Lane 2 and 15 contain molecular weight markers; and Lanes 3-14 represent leptin samples at day 1-12, respectively.

Figure 8 depicts the *in vivo* bioactivity for a Zn:leptin-containing hydrogel (PLGA/PEG (74%/26% w/w)) formulation. -✱- depicts a 20mM acetate, pH 4.8, buffer control, 100µl on day 0; -•- depicts Zn:leptin, 100 mg/kg, 100µl on day 0; and -■- depicts a Zn:leptin-containing hydrogel (PLGA/PEG (74%/26% w/w)), 20 mg/mL leptin, 100 mg/kg, 100µl on day 0. % body weight change (from the day 0 body weight) is plotted vs. time (days).

DETAILED DESCRIPTION OF THE INVENTION

As used herein, the following terms shall have the following meaning:

"Reverse thermal gelation" is defined as meaning the temperature below which a copolymer is soluble in water and above which the block copolymer forms a semi-solid, i.e. gels, emulsions, dispersions and suspensions.

"LCST", or lower critical solution temperature, is defined as meaning the temperature at which a biodegradable block copolymer undergoes reverse thermal gelation. For purposes of the present invention, the term "LSCT" can be used interchangeably with "reverse thermal gelation temperature".

"Depot" is defined as meaning a drug delivery liquid which, following injection into a warm blooded animal, has formed a gel upon having the temperature raised to or above the LCST.

"Biodegradable" is defined as meaning that the block copolymer will erode or degrade *in vivo* to form smaller non-toxic components.

"Parenteral administration" is defined as meaning any route of administration other than the alimentary canal, including, for example, subcutaneous and intramuscular.

The present invention involves utilization of block copolymers having hydrophobic ("A") block segments and hydrophilic ("B") block segments. The block copolymers are triblock copolymers, e.g., ABA or BAB type block copolymers, which possess reverse thermal gelation properties and are biodegradable and biocompatible. Importantly, triblock copolymers of the present invention provide instant gelation and possess the necessary rate of degradation to be commercially useful.

Biodegradable hydrophobic A block segments contemplated for use include poly(α-hydroxy acid) members derived from or selected from the group consisting of homopolymers and copolymers of poly(lactide)s (d,l- or l- forms), poly(glycolide)s, polyanhydrides, polyorthoesters, polyetheresters, polycaprolactone, polyesteramides, polycarbonate, polycyanoacrylate, polyurethanes, polyacrylate, blends and copolymers thereof.

The term "PLGA" as used herein is intended to refer to a polymer of lactic acid alone, a polymer of glycolic acid alone, a mixture of such polymers, a copolymer of glycolic acid and lactic acid, a mixture of such copolymers, or a mixture of such polymers and copolymers. Preferably, the biodegradable A block polymer will be poly lactide-co-glycolide (PLGA), and the PLGA composition will be such that the necessary rate of gelation and rate of degradation are obtained.

The range of molecular weights contemplated for the polymers to be used in the present processes can be readily determined by a person skilled in the art based upon such factors the desired polymer degradation rate. Typically, the range of molecular weight for the A block will be 1000 to 20,000 Daltons.

Hydrophilic B block segments contemplated for use include polyethylene glycols having average molecular weights of between about 500 and 10,000.

The copolymer compositions for the block copolymers of the present invention are specially regulated to assure retention of the desired water-solubility and gelling properties, i.e., the ratios must be such that the block copolymers possess water solubility at temperatures below the LCST, and such that there is instant gelation under physiological conditions (i.e. pH 7.0 and 37°C) so as to minimize the

initial burst of drug. In the hydrogels of the present invention the hydrophobic A block makes up 55% to 90% by weight of the copolymer and the hydrophilic B block makes up 10% to 45% of the copolymer.

The concentration at which the block copolymers of the present invention remain soluble below the LCST are generally up to about 60% by weight, with 10%-30% preferred. The concentration utilized will depend upon the copolymer composition actually used, as well as whether or not a gel or emulsion is desired.

The thermosensitive block copolymers of the present invention can be prepared by thermal condensation. In a typical experiment, A-B-A block copolymers of PLGA/PLA (block A) and PEG (block B) are synthesized by mixing either homopolymer of poly lactide (PLA) or copolymer of poly lactide-co-gycolide (PLGA) with polyethylene glycol (PEG) and allowing di-hydroxy PEG to react with PLGA or PLA at 160°C under reduced pressure. Different weight ratios of PLGA and PEG were used for thermal condensation to obtain a series of block copolymers with desirable copolymer composition and block lengths. Copolymer composition and relative block lengths were confirmed by ^{1}H-NMR spectroscopy.

Alternatively, the copolymers could be synthesized in a melt process which involves ring opening polymerization of A block using B block as the initiator. In a typical experiment, the ABA triblock copolymer is prepared by stannous octoate catalyzed ring-opening polymerization of d,l-dilactide (or PLGA) using α,ω-dihydroxy-terminated PEG as the initiator. The mole ratio of B block to d,l-dilactide (or PLGA) is used to control the lengths of the A blocks, and provide a series of polymers with increasing A block

contents and hydrophobicites. The relative A and B block lengths can be confirmed by ^{1}H-NMR spectroscopy.

The process used to mix the copolymers with a biologically active agent and/or other materials involves dissolving the ABA block copolymers in an aqueous solution, followed by addition of the biologically active agent (in solution, suspension or powder), followed by thorough mixing to assure a homogeneous mixing of the biologically active agent throughout the copolymer. Alternatively, the process can involve the dissolving of the ABA block copolymer in a biologically active agent-containing solution. In either case, the process is conducted at a temperature lower than the gelation temperature of the copolymer and the material is implanted into the body as a solution which then gels or solidifies into a depot in the body. In the compositions of the present invention, the biologically active agent will generally have a concentration in the range of 0 to 200 mg/mL.

Buffers contemplated for use in the preparation of the biologically active agent-containing hydrogels are buffers which are all well known by those of ordinary skill in the art and include sodium acetate, Tris, sodium phosphate, MOPS, PIPES, MES and potassium phosphate, in the range of 25mM to 500mM and in the pH range of 4.0 to 8.5.

It is also envisioned that other excipients, e.g., various sugars, salts, or surfactants, may be included in the biologically active agent-containing hydrogels of the present invention in order to alter the LCST or rate of gelation of the gels. The ability to alter the rate of gelation and/or LCST is important and an otherwise non-useful hydrogel may be made useful by addition of such excipients. Examples of such sugars include glucose or sucrose in the range of 5% to

20%. Examples of such salts include sodium chloride or zinc chloride in the range of 0.5% to 10%.

As used herein, biologically active agents refers to recombinant or naturally occurring proteins, whether human or animal, useful for prophylactic, therapeutic or diagnostic application. The biologically active agent can be natural, synthetic, semi-synthetic or derivatives thereof. In addition, biologically active agents of the present invention can be perceptible. A wide range of biologically active agents are contemplated. These include but are not limited to hormones, cytokines, hematopoietic factors, growth factors, antiobesity factors, trophic factors, anti-inflammatory factors, small molecules and enzymes (see also U.S. Patent No. 4,695,463 for additional examples of useful biologically active agents). One skilled in the art will readily be able to adapt a desired biologically active agent to the compositions of present invention.

Proteins contemplated for use would include but are not limited to interferon consensus (see, U.S. Patent Nos. 5,372,808, 5,541,293 4,897,471, and 4,695,623 hereby incorporated by reference including drawings), interleukins (see, U.S. Patent No. 5,075,222, hereby incorporated by reference including drawings), erythropoietins (see, U.S. Patent Nos. 4,703,008, 5,441,868, 5,618,698 5,547,933, and 5,621,080 hereby incorporated by reference including drawings), granulocyte-colony stimulating factors (see, U.S. Patent Nos. 4,810,643, 4,999,291, 5,581,476, 5,582,823, and PCT Publication No. 94/17185, hereby incorporated by reference including drawings), stem cell factor (PCT Publication Nos. 91/05795, 92/17505 and 95/17206, hereby incorporated by reference including drawings), and leptin (OB protein) (see

PCT publication Nos. 96/40912, 96/05309, 97/00128, 97/01010 and 97/06816 hereby incorporated by reference including figures).

It is desirable to have such protein containing sustained-release compositions as such compositions could serve to enhance the effectiveness of either exogenously administered or endogenous protein, or could be used, for example, to reduce or eliminate the need for exogenous protein administration.

Moreover, because the materials utilized in the present invention are biocompatible and biodegradable, use of the protein compositions of the present invention help prevent adverse injection site reactions sometimes associated with i.v. injections of various proteins such as leptin.

In addition, biologically active agents can also include insulin, gastrin, prolactin, adrenocorticotropic hormone (ACTH), thyroid stimulating hormone (TSH), luteinizing hormone (LH), follicle stimulating hormone (FSH), human chorionic gonadotropin (HCG), motilin, interferons (alpha, beta, gamma), tumor necrosis factor (TNF), tumor necrosis factor-binding protein (TNF-bp), interleukin-1 receptor antagonist (IL-1ra), brain derived neurotrophic factor (BDNF), glial derived neurotrophic factor (GDNF), neurotrophic factor 3 (NT3), fibroblast growth factors (FGF), neurotrophic growth factor (NGF), bone growth factors such as osteoprotegerin (OPG), insulin-like growth factors (IGFs), macrophage colony stimulating factor (M-CSF), granulocyte macrophage colony stimulating factor (GM-CSF), megakaryocyte derived growth factor (MGDF), keratinocyte growth factor (KGF), thrombopoietin, platelet-derived growth factor (PGDF), colony simulating growth factors (CSFs), bone

morphogenetic protein (BMP), superoxide dismutase (SOD), tissue plasminogen activator (TPA), urokinase, streptokinase and kallikrein. The term proteins, as used herein, includes peptides, polypeptides, consensus molecules, analogs, derivatives or combinations thereof.

Also included are those polypeptides with amino acid substitutions which are "conservative" according to acidity, charge, hydrophobicity, polarity, size or any other characteristic known to those skilled in the art. *See generally*, Creighton, Proteins, W.H. Freeman and Company, N.Y., (1984) 498 pp. plus index, *passim*. One may make changes in selected amino acids so long as such changes preserve the overall folding or activity of the protein. Small amino terminal extensions, such as an amino-terminal methionine residue, a small linker peptide of up to about 20-25 residues, or a small extension that facilitates purification, such as a poly-histidine tract, an antigenic epitope or a binding domain, may also be present. *See*, in general, Ford et al., *Protein Expression and Purification* 2:95-107 (1991), which is herein incorporated by reference. Polypeptides or analogs thereof may also contain one or more amino acid analogs, such as peptidomimetics.

In general, comprehended by the invention are pharmaceutical compositions comprising effective amounts of chemically modified protein, or derivative products, together with pharmaceutically acceptable diluents, preservatives, solubilizers, emulsifiers, adjuvants and/or carriers needed for administration. (See PCT 97/01331 hereby incorporated by reference.) The optimal pharmaceutical formulation for a desired biologically active agent will be determined by one

skilled in the art depending upon the route of administration and desired dosage. Exemplary pharmaceutical compositions are disclosed in Remington's Pharmaceutical Sciences (Mack Publishing Co., 18th Ed., Easton, PA, pgs. 1435-1712 (1990)).

The pharmaceutical compositions of the present invention are administered as a liquid via intramuscular or subcutaneous route and undergo a phase change wherein a gel is formed within the body, since the body temperature will be above the gelation temperature of the material. The release rates and duration for the particular biologically active agents will be a function of, *inter alia*, hydrogel density and the molecular weight of the agent.

Therapeutic uses of the compositions of the present invention depend on the biologically active agent used. One skilled in the art will readily be able to adapt a desired biologically active agent to the present invention for its intended therapeutic uses. Therapeutic uses for such agents are set forth in greater detail in the following publications hereby incorporated by reference including drawings. Therapeutic uses include but are not limited to uses for proteins like interferons (*see*, U.S. Patent Nos. 5,372,808, 5,541,293, hereby incorporated by reference including drawings), interleukins (*see*, U.S. Patent No. 5,075,222, hereby incorporated by reference including drawings), erythropoietins (*see*, U.S. Patent Nos. 4,703,008, 5,441,868, 5,618,698 5,547,933, and 5,621,080 hereby incorporated by reference including drawings), granulocyte-colony stimulating factors (*see*, U.S. Patent Nos. 4,999,291, 5,581,476, 5,582,823, 4,810,643 and PCT Publication No. 94/17185, hereby incorporated by reference including drawings), stem cell factor (PCT Publication Nos. 91/05795, 92/17505

and 95/17206, hereby incorporated by reference including drawings), and the OB protein (*see* PCT publication Nos. 96/40912, 96/05309, 97/00128, 97/01010 and 97/06816 hereby incorporated by reference including figures). In addition, the present compositions may also be used for manufacture of one or more medicaments for treatment or amelioration of the conditions the biologically active agent is intended to treat.

In the sustained-release compositions of the present invention, an effective amount of active ingredient will be utilized. As used herein, sustained release refers to the gradual release of active ingredient from the polymer matrix, over an extended period of time. The sustained release can be continuous or discontinuous, linear or non-linear, and this can be accomplished using one or more polymer compositions, drug loadings, selection of excipients, or other modifications. The sustained release will result in biologically effective serum levels of the active agent (typically above endogenous levels) for a period of time longer than that observed with direct administration of the active agent. Typically, a sustained release of the active agent will be for a period of a week or more, preferably up to one month.

The following examples are offered to more fully illustrate the invention, but are not to be construed as limiting the scope thereof.

Materials

Low molecular weight (Mn 2000-6000) PLGA (poly Lactic acid-co-Glycolic acid) and PLA (poly Lactic acid) were synthesized by direct thermal condensation of glycolic acid and lactic acid at 180°C under reduced pressure. High molecular weight PLGAs were obtained from B.I. Chemicals. Polyethylene glycols (PEG) were obtained from Fluka Chemicals. Leptin, zinc-leptin, GCSF, Fc-Leptin, and Fc-OPG were obtained from Amgen Inc. All other chemicals are from sources well known in the art.

Example 1

This example describes synthesis of a PLGA/PEG, A-B-A (PLGA-PEG-PLGA), block copolymer by thermal condensation. The thermal condensation method is generally depicted in Figure 1.

30 g PLGA (75%/25% LA/GA ratio) (Mn 3740, MW 7050) and 10.7 g polyethylene glycol (MW 1000) were placed into a three-neck round bottom flask equipped with a thermometer, a nitrogen gas inlet, and a distillation condenser connected to a vacuum pump. After addition of the polymers, the temperature of the reaction mixture was raised slowly to 160°C under nitrogen purging. The condensation reaction was further carried out at 160°C for 14 hours under 500 millitorr pressure and with continuous bubbling of dry nitrogen gas. At the end of the condensation reaction, the reaction mixture was cooled, dissolved in methylene chloride and precipitated with an excess of cold isopropanol.

The isolated polymer was dried at 40°C under vacuum for 48 hours. The molecular weight of the block copolymer was determined by gel permeation chromatography (GPC) using polystyrene standards. The copolymer

composition and relative block lengths were determined by ^{1}H-NMR.

The PLGA/PEG block copolymer dissolved either in 100mM sodium acetate, pH 6.0, or 100mM sodium phosphate, pH 7.0, exhibited a unique thermoreversible property (solution below room temperature and gel above room temperature, sol-gel-sol) with lower critical solution temperature (LCST) at about 30°C to 35°C.

Example 2

This example describes the synthesis of PLGA/PEG, A-B-A (PLGA-PEG-PLGA), block copolymers using PLGA with different lactic acid to glycolic acid ratios.

The synthesis and characterization procedures described in Example 1 were utilized to prepare PLGA/PEG block copolymers using PLGA with different LA to GA ratios (see Table 1 below). The block copolymers listed below showed thermoreversibility (Sol-gel-sol) with LCST at about 30°C to 35°C.

Table 1

PLGA (LA/GA ratio) wt (g)	PEG 1000 wt (g)	PLGA/PEG wt ratio	PLGA/PEG calculated	Molar ratio by NMR
PLA (100%) 45g (Mn 3480, MW 6590)	17.55g	72/28	1.56	1.48
PLGA (75/25%) 30g (Mn 3740, MW 7090)	11.74g	72/28	1.65	1.56
PLGA (50/50%) 30g (Mn 3480/MW 6590)	10.71g	74/26	1.8	1.78
PLGA (56/44%) 40g (Mn 3480/MW 6590)	15.60g	72/28	1.71	1.66

Example 3

This example describes the synthesis of PLGA/PEG, A-B-A (PLGA-PEG-PLGA), block copolymers using different weight ratios of PLGA and PEG.

The synthesis and characterization procedure described in Example 1 were utilized to prepare PLGA/PEG block copolymers with various PLGA to PEG ratios (see Table 2 below). All of the block copolymers listed below showed thermoreversibility (sol-gel-sol) with LCST in the range of 25°C - 35°C.

Table 2

PLGA (75/25) Mn 3740,MW 7090	PEG 1000 wt (g)	PLGA/PEG wt ratio	PLGA/PEG calculated	Molar ratio by NMR
30g	9.47g	76/24	2.03	
40g	14.28g	74/26	1.79	1.70
40g	14.90g	73/27	1.72	1.65
30g	11.84g	72/28	1.65	1.56
30g	12.63g	70/30	1.53	1.50
30g	14.21g	68/32	1.36	1.32
30g	15.48g	66/34	1.24	1.17
30g	16.70g	64/36	1.15	1.08
30g	18.40g	62/38	1.05	

Example 4

This example describes the preparation of a leptin/hydrogel formulation and the methods used to determine the *in vitro* release kinetics, *in vivo* release kinetics, and pharmacokinetics of the leptin/hydrogel.

Preparation of leptin/hydrogel formulation

The PLGA/PEG block copolymer described in Example 1 was dissolved in 50mM sodium acetate, pH 6.0. Leptin solution (formulated in 20mM acetate, pH 4.8) was slowly added to the hydrogel solution and the mixture was gently swirled on an orbital shaker at 5°C to assure a homogeneous mixing of leptin throughout the hydrogel solution. The final concentration of the copolymer in the final leptin/hydrogel formulation was 10-50% (w/w) and the leptin concentration was in the range of 0-100 mg/ml. The final leptin/hydrogel formulation was filtered through 0.2μ filter and stored either as a solution at 5°C or stored as a frozen mass at -20°C.

Alternatively, the leptin/hydrogel formulation was prepared by dissolving the PLGA/PEG block copolymer in a leptin solution. The leptin solution concentration was varied to obtain desirable copolymer as well as the desired protein concentration in final formulation.

In vitro Release Study

The *in vitro* release of leptin from the leptin/hydrogel was carried out in 20mM sodium phosphate, 5% sorbitol, pH 7.4, at 37°C. 1 ml of leptin/hydrogel solution formulation was placed in a glass vial at 37°C. Upon gelation of the leptin/hydrogel formulation, 1 ml of 20mM phosphate, 5% sorbitol, pH 7.4, buffer was added directly above and in contact with the gel. The amount of leptin released in the top buffer phase was determined by UV spectrophotometer at 280nm as well as by SEC-HPLC at 220nm. To maintain a perfect sink condition the aqueous receptor phase above the gel was completely

removed at definite time intervals and replaced by fresh buffer. The % leptin released over time is depicted in Figure 2. The integrity of the leptin released from the hydrogel formulation was confirmed by HPLC (data not shown) and gel electrophoresis (SDS-PAGE) (see Figure 3).

***In vivo* bioactivity**

The *in vivo* bioactivity of leptin/hydrogel formulations were evaluated in normal mice. Mice were injected subcutaneously (s.c.) with either: a) 0.1 ml of 20mM acetate buffer, pH 4.8, (n=5, day 0 only); (b) 0.1 ml of 20 mg/ml leptin formulated in 20mM acetate buffer, pH 4.8 (n=5, 100 mg/kg, day 0 only); (c) 0.1 ml of 2 mg/ml leptin formulated in 20mM acetate buffer, pH 4.8 (n=5, 10 mg/kg, daily); (d) 0.1 ml of a leptin/hydrogel (74/26% (PLGA/PEG)(w/w)) formulation consisting of 20 mg/ml leptin, in 20mM acetate, pH 4.8 (n=5, 100 mg/kg, day 0 only); (e) 0.2 ml of a leptin/hydrogel (74/26% (PLGA/PEG)(w/w)) formulation consisting of 20 mg/ml leptin, in 20mM acetate, pH 4.8 (n=5, 200 mg/kg ,day 0 only); or (f) 0.1 ml of a hydrogel (74/26% (PLGA/PEG)(w/w)) control, formulated in 50mM acetate, pH 6.0 (n=5, day 0 only).

% body weight change (from the day 0 body weight) was determined by weighing the animals daily until the body weight of the animals injected with sample (b), (d) and (e) reached the body weights of the animals injected with buffer control (sample (a)). Importantly, a single s.c. injection of 100 mg/kg leptin/hydrogel formulation (sample (d)) showed sustained weight loss in normal mice over a 10 day period. The duration of sustained weight loss effect was further extended up to 14 days when the dose was

increased to 200 mg/kg (sample (e)). It was also observed that a single injection of 100 mg/kg or 200 mg/kg leptin/hydrogel at day '0' was more efficacious up to 14 days than daily injections of 10 mg/kg leptin without hydrogel. These results are depicted in Figure 4.

Pharmacokinetics Study

A pharmacokinetics study was carried out in male rats. After a single s.c. injection of either: 1) 100 mg/kg dose of leptin (20 mg/ml) formulated in 20mM acetate buffer, pH 4.8); or 2) a leptin/hydrogel (74/26% (PLGA/PEG)(w/w)) formulation consisting of 20 mg/ml leptin, in 20mM acetate, pH 4.8, blood samples were collected at various time intervals and analyzed for leptin by ELISA assay. As shown in Figure 5, serum concentrations of leptin were detectable for up to 168 hours for animals injected with the leptin/hydrogel formulation.

Example 5

This example describes the incorporation of G-CSF into the hydrogel and the results of *in vitro* release studies using the formulation.

GCSF solution (formulated in 10mM acetate, 5% sucrose, pH 4.0) was added to the copolymer hydrogel solution (formulated in 20mM acetate, pH 6.0) as described in Example 4. The final concentration of the copolymer in the GCSF/hydrogel formulation was 10-50% (w/w) and the GCSF concentration was in the range of 1-20 mg/ml. The *in vitro* release of GCSF from the hydrogel was carried out in 20mM sodium phosphate buffer, pH 7.4, at 37°C as described in Example 4.

The % GCSF released over time is shown in Figure 6. As depicted in Figure 6, nearly 100% of the GCSF is released over a 9-10 day period of time. The integrity of the GCSF released from the hydrogel formulation was confirmed by HPLC (data not shown) and gel electrophoresis (SDS-PAGE)(see Figure 7).

Example 6

This example describes the incorporation of an Fc-OPG protein into the hydrogel and the results of *in vitro* release studies using the Fc-OPG/hydrogel formulation.

The Fc-OPG/hydrogel formulation was prepared as described in Example 4 by adding Fc-OPG solution (formulated in 10mM sodium acetate, 5% sorbitol, 0.02 mg/ml tween 20, pH 5.0) to the copolymer solution (formulated in 50mM acetate, pH 6.0). The *in vitro* release of Fc-OPG from the hydrogel was carried out in 20mM sodium phosphate buffer, pH 7.4, at 37°C as described in Example 4. The % Fc-OPG released over time is shown in Figure 6. As depicted in Figure 6, nearly 100% of the Fc-OPG is released over a 8-9 day period of time.

Example 7

This example describes incorporation of the Zn:leptin suspension into PLGA/PEG hydrogel and the results of *in vivo* release kinetics of the leptin from the Zn:leptin/hydrogel.

The PLGA/PEG block polymers described in the examples above were hydrated in 100mM Tris, pH 8.0 buffer. The final pH of the hydrogel solution was maintained between 6.5 - 7.0 and then a zinc chloride

solution was added to the hydrogel to obtain a 0.1mM $ZnCl_2$ concentration in the final hydrogel solution. To this hydrogel solution, a Zn:leptin suspension was added as described in Example 4. The final Zn:leptin concentration in the hydrogel described in this example was 20 mg/ml. The *in vivo* bioactivity of a Zn:leptin/hydrogel (74/26% (PLGA/PEG)(w/w)) formulation was carried out as described in Example 4. The results of the *in vivo* bioactivity studies are depicted in Figure 8.

Example 8

This example describes the incorporation of Zn:GCSF into the PLGA/PEG hydrogel and the results of *in vitro* release studies using the formulation.

The PLGA/PEG block copolymer described in the examples above was hydrated in 100mM PIPES, pH 7.5 buffer. The final pH of the hydrogel solution was maintained between 6.5 - 7.0 and then a zinc chloride solution was added to the hydrogel to obtain a 0.1mM $ZnCl_2$ concentration in the final hydrogel solution. To this hydrogel solution, a Zn:GCSF suspension was added as described in Example 4. The *in vitro* release of GCSF from the hydrogel was carried out in 20mM sodium phosphate buffer, pH 7.4, at 37°C, as described in Example 4. It was demonstrated that sustained release of GCSF could be obtained from these hydrogel formulations.

Example 9

This example describes the incorporation of GCSF-crystals in the PLGA/PEG hydrogel and the results of *in vitro* release studies using the formulation.

The block polymer described in the examples above was hydrated in 100mM MES, pH 7.5 buffer. The final pH of the hydrogel solution was maintained between 6.5 - 7.0 and then a $MgCl_2$ solution was added to the hydrogel to obtain a 0.2M $MgCl_2$ concentration in the final hydrogel solution. To this hydrogel solution, a GCSF crystals suspension was added as described in Example 4. The *in vitro* release of GCSF from the hydrogel was carried out in 20mM sodium phosphate buffer, pH 7.4, at 37°C, as described in Example 4. It was demonstrated that sustained release of GCSF could be obtained from these hydrogel formulations.

Example 10

This example describes the effect of various excipients on the LCST of PLGA/PLGA, A-B-A block copolymers. As indicated in Table 4 below, the addition of various sugars, salts, surfactants, etc. can effect the rate of gelation and LCST of the hydrogels.

Table 4

Excipient Added	Effect on Gelation	Effect on LCST
Sugars at 5%-20% (e.g. glucose, sucrose)	↑ rate of gelation Firm gel	Lowered LCST
Salts at 0.5%-10% (e.g. $NaCl_,$, $ZnCl_,$,Na_2SO_4)	↑ rate of gelation Firm gel	Lowered LCST
Surfactants (e.g. Tween, SDS)	↓ rate of gelation Soft gel	-----
Glycerol at 2%-10% (e.g. $NaCl_,$, $ZnCl_,$,Na_2SO_4)	↑ rate of gelation Firm gel	Lowered LCST
Polyethylene glycol at 5%-20%	↓ rate of gelation Soft gel	Increased LCST

The present invention has been described in terms of particular embodiments found or proposed to comprise preferred modes for the practice of the

invention. It will be appreciated by those of ordinary skill in the art that, in light of the present disclosure, numerous modifications and changes can be made in the particular embodiments exemplified without departing from the intended scope of the invention.

WHAT IS CLAIMED IS:

1. A pharmaceutical composition for the sustained administration of an effective amount of a biologically active agent, or a derivative, analog, fusion, conjugate, or chemically modified form thereof, comprising an injectable biodegradable polymeric matrix into which said biologically active agent has been incorporated, said polymeric matrix having reverse thermal gelation properties, and wherein said injectable polymeric matrix is maintained at a temperature below the lower critical solution temperature of said polymeric matrix.

2. The composition of claim 1, wherein said polymeric matrix is a biodegradable block copolymer comprising:

(a) 55% to 90% by weight of a hydrophobic A polymer block and;

(b) 10% to 45% by weight of a hydrophilic B polymer block comprising a polyethylene glycol having an average molecular weight of between 500-10000.

3. The composition of claim 2, wherein said hydrophobic A polymer block is a poly(α-hydroxy acid) having an average molecular weight of between 1000-20,000.

4. The composition of claim 3, wherein said poly(α-hydroxy acid) is selected from the group consisting of poly(lactide)s (d,l- or l- forms), poly(glycolide)s, polyanhydrides, polyorthoesters, polyetheresters, polycaprolactone, polyesteramides,

polycarbonate, polycyanoacrylate, polyurethanes, polyacrylate, blends and copolymers thereof.

5. The composition of claim 4, wherein said poly(α-hydroxy acid) is poly lactide-co-glycolide (PLGA).

6. The composition of claim 5, wherein said block copolymer is a triblock copolymer having a configuration selected from the group consisting of ABA or BAB block segments.

7. The composition of claim 6, wherein said hydrophobic A polymer block comprises 74% by weight of said block copolymer and said hydrophilic B polymer block comprises 26% by weight of said block copolymer.

8. The composition of claim 7 further comprising an excipient which will vary the lower critical solution temperature and increase the rate of gelation of said block copolymer.

9. The composition of claim 1, wherein said biologically active agent is a protein selected from the group consisting of interferon consensus, interleukins, erythropoietins, granulocyte-colony stimulating factor (GCSF), stem cell factor (SCF), leptin (OB protein), interferons (alpha, beta, gamma), tumor necrosis factor (TNF), tumor necrosis factor-binding protein (TNF-bp), interleukin-1 receptor antagonist (IL-1ra), brain derived neurotrophic factor (BDNF), glial derived neurotrophic factor (GDNF), neurotrophic factor 3 (NT3), fibroblast growth factors (FGF), neurotrophic growth factor (NGF), bone growth factors such as osteoprotegerin (OPG), granulocyte

macrophage colony stimulating factor (GM-CSF), megakaryocyte derived growth factor (MGDF), keratinocyte growth factor (KGF), thrombopoietin, platelet-derived growth factor (PGDF), tissue plasminogen activator (TPA), urokinase, streptokinase and kallikrein.

10. The composition of claim 1, wherein said biologically active agent is a small molecule.

11. A method for the parenteral administration of a biologically active agent, or a derivative, analog, fusion, conjugate, or chemically modified form thereof, in a biodegradable polymeric matrix to a warm blooded animal with the resultant sustained release of said agent concomitant with biodegradation of said polymeric matrix, which comprises:

(a) providing an injectable liquid polymeric matrix comprising a biodegradable block copolymer having reverse thermal gelation properties, and into which a biologically active agent has been incorporated;

(b) maintaining said liquid polymeric matrix at a temperature below the lower critical solution temperature of said polymeric matrix; and

(c) injecting said liquid parenterally into said animal, thus forming a gel depot of said drug and polymeric matrix as the temperature of said liquid is raised in the body of said animal above the lower critical solution temperature of the polymeric matrix.

12. The method of claim 11, wherein said polymeric matrix is a biodegradable block copolymer comprising:

(a) 55% to 90% by weight of a hydrophobic A polymer block and;

(b) 10% to 45% by weight of a hydrophilic B polymer block comprising a polyethylene glycol having an average molecular weight of between 500-10000.

13. The method of claim 12, wherein said hydrophobic A polymer block is a poly(α-hydroxy acid) having an average molecular weight of between 1000-20,000.

14. The method of claim 13, wherein said poly(α-hydroxy acid) is poly lactide-co-glycolide (PLGA).

15. The method of claim 14, wherein said block copolymer is a triblock copolymer having a configuration selected from the group consisting of ABA or BAB block segments.

16. The method of claim 15, wherein said hydrophobic A polymer block comprises 74% by weight of said block copolymer and said hydrophilic B polymer block comprises 26% by weight of said block copolymer.

17. The method of claim 16 further comprising an excipient which will vary the lower critical solution temperature and increase the rate of gelation of said block copolymer.

18. The method of claim 11, wherein said biologically active agent is a protein selected from the group consisting of interferon consensus, interleukins, erythropoietins, granulocyte-colony

stimulating factor (GCSF), stem cell factor (SCF), leptin (OB protein), interferons (alpha, beta, gamma), tumor necrosis factor (TNF), tumor necrosis factor-binding protein (TNF-bp), interleukin-1 receptor antagonist (IL-1ra), brain derived neurotrophic factor (BDNF), glial derived neurotrophic factor (GDNF), neurotrophic factor 3 (NT3), fibroblast growth factors (FGF), neurotrophic growth factor (NGF), bone growth factors such as osteoprotegerin (OPG), granulocyte macrophage colony stimulating factor (GM-CSF), megakaryocyte derived growth factor (MGDF), keratinocyte growth factor (KGF), thrombopoietin, platelet-derived growth factor (PGDF), tissue plasminogen activator (TPA), urokinase, streptokinase and kallikrein.

19. The method of claim 11, wherein said biologically active agent is a small molecule.

FIG. 1

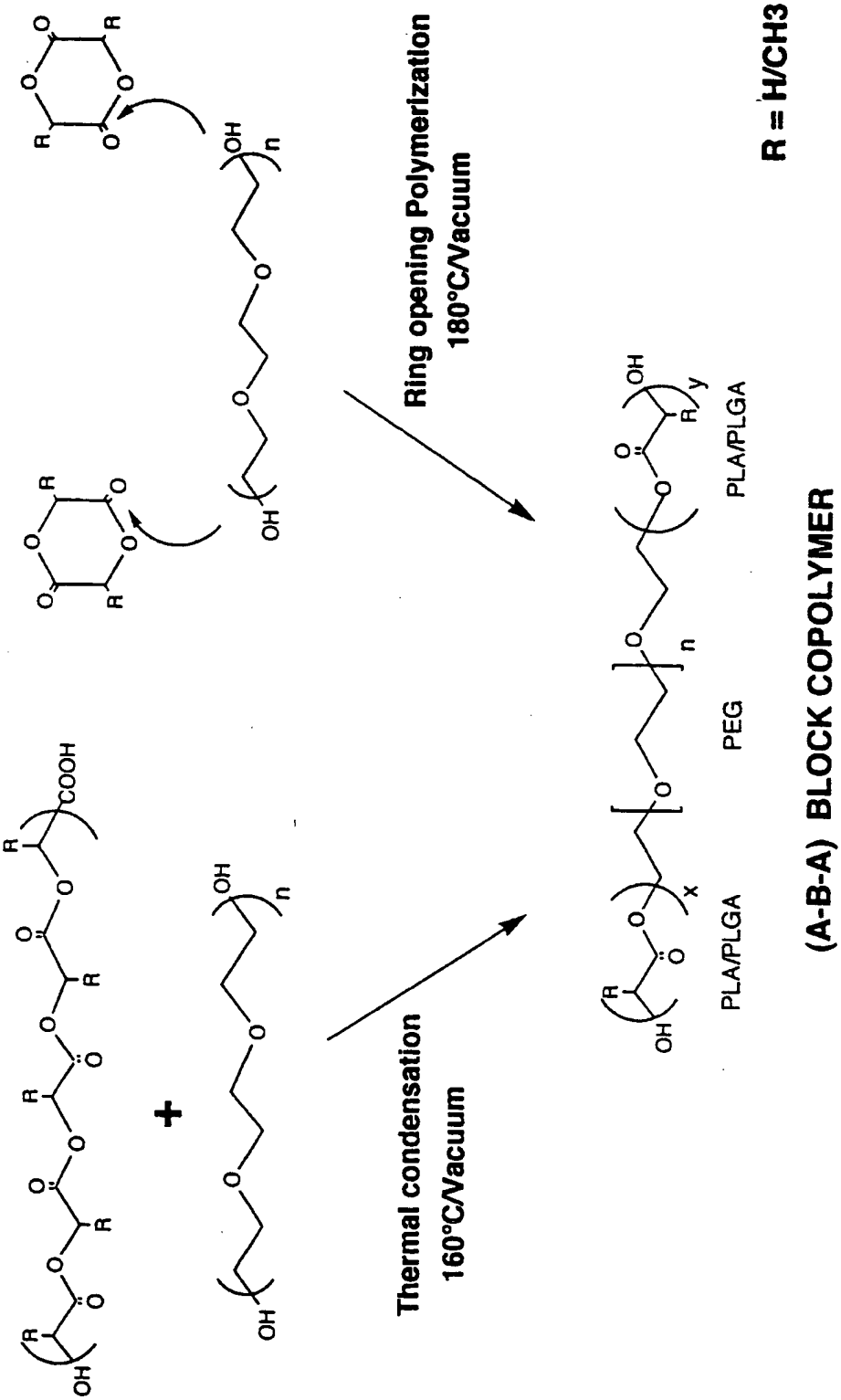

Ring opening Polymerization
180°C/Vacuum
Thermal condensation
160°C/Vacuum
R = H/CH3
PLA/PLGA
PEG
PLA/PLGA
(A-B-A) BLOCK COPOLYMER

FIG. 2

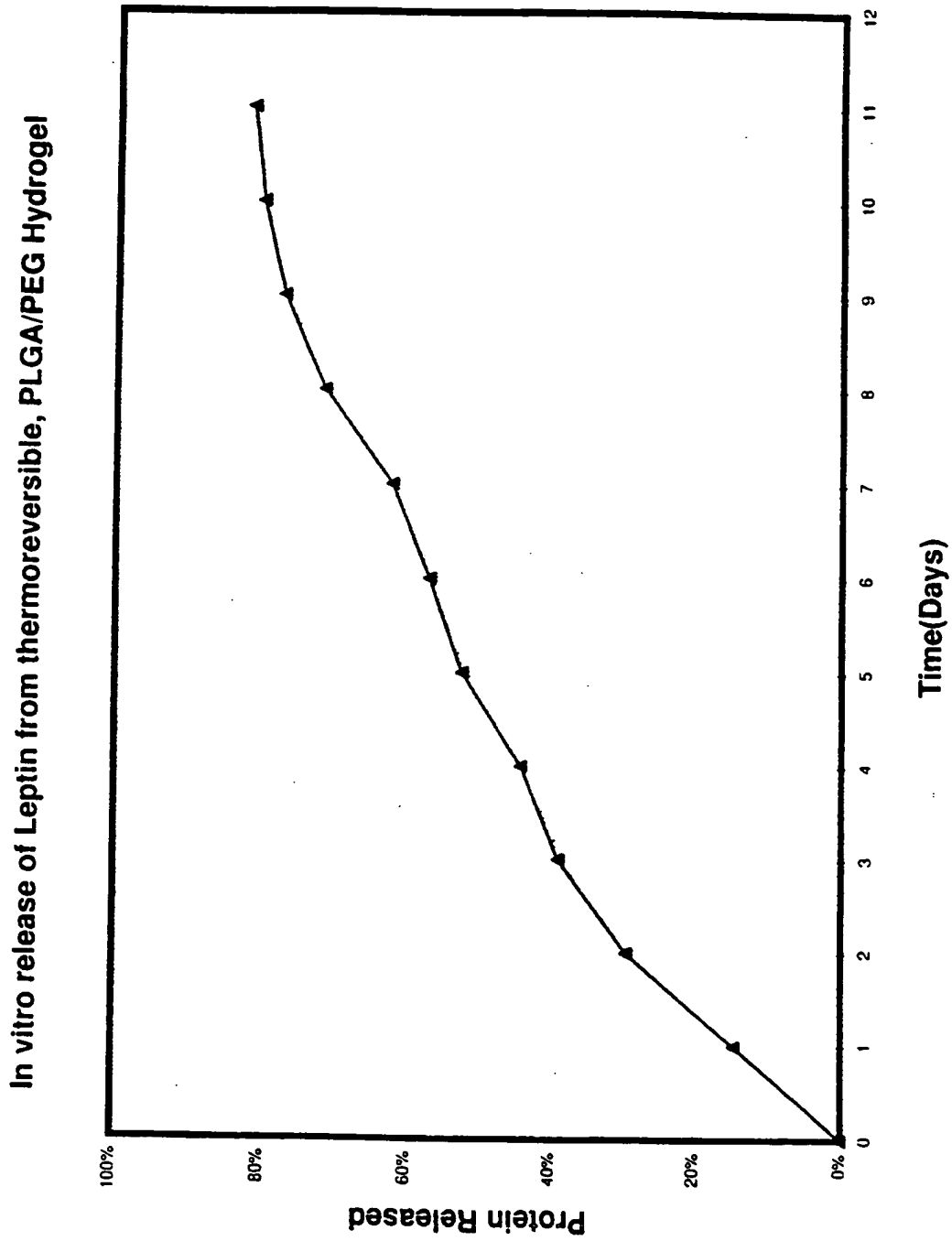

In vitro release of Leptin from thermoreversible, PLGA/PEG Hydrogel
Protein Released
100%
80%
60%
40%
20%
0%
Time(Days)
0
1
2
3
4
5
6
7
8
9
10
11
12

FIG. 3

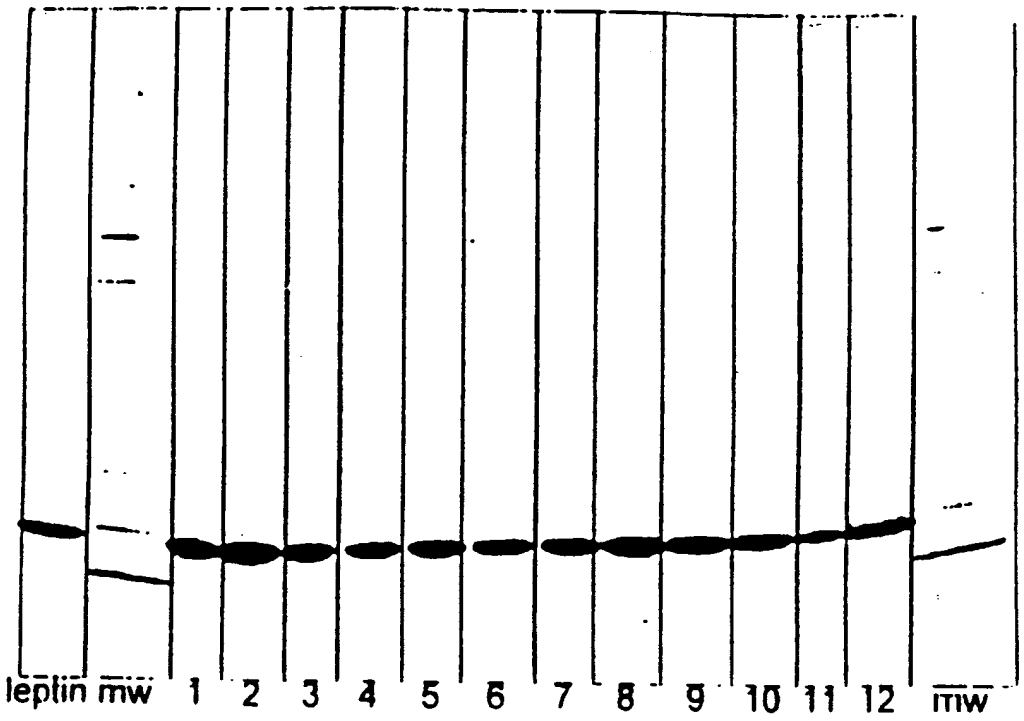


FIG. 4

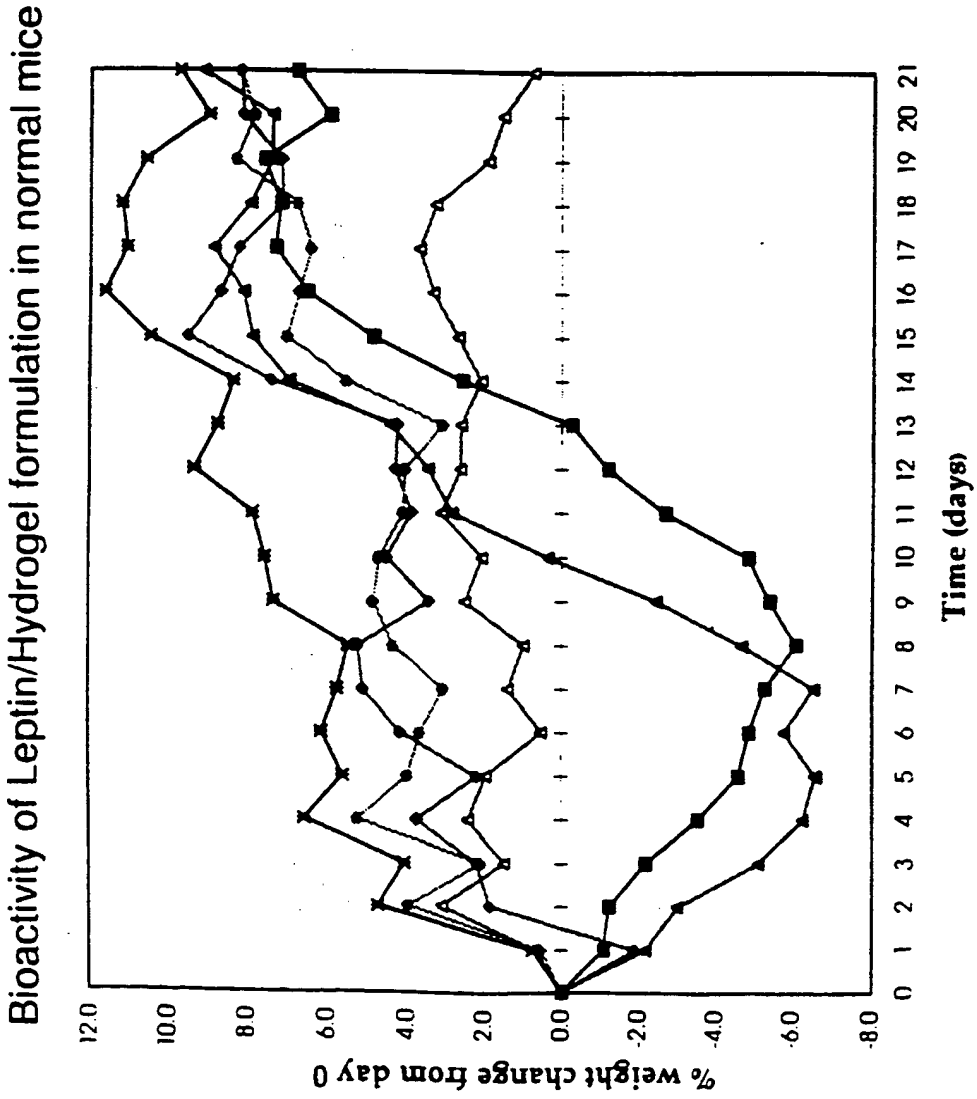

Bioactivity of Leptin/Hydrogel formulation in normal mice
% weight change from day 0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
-2.0
-4.0
-6.0
-8.0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Time (days)

FIG. 5

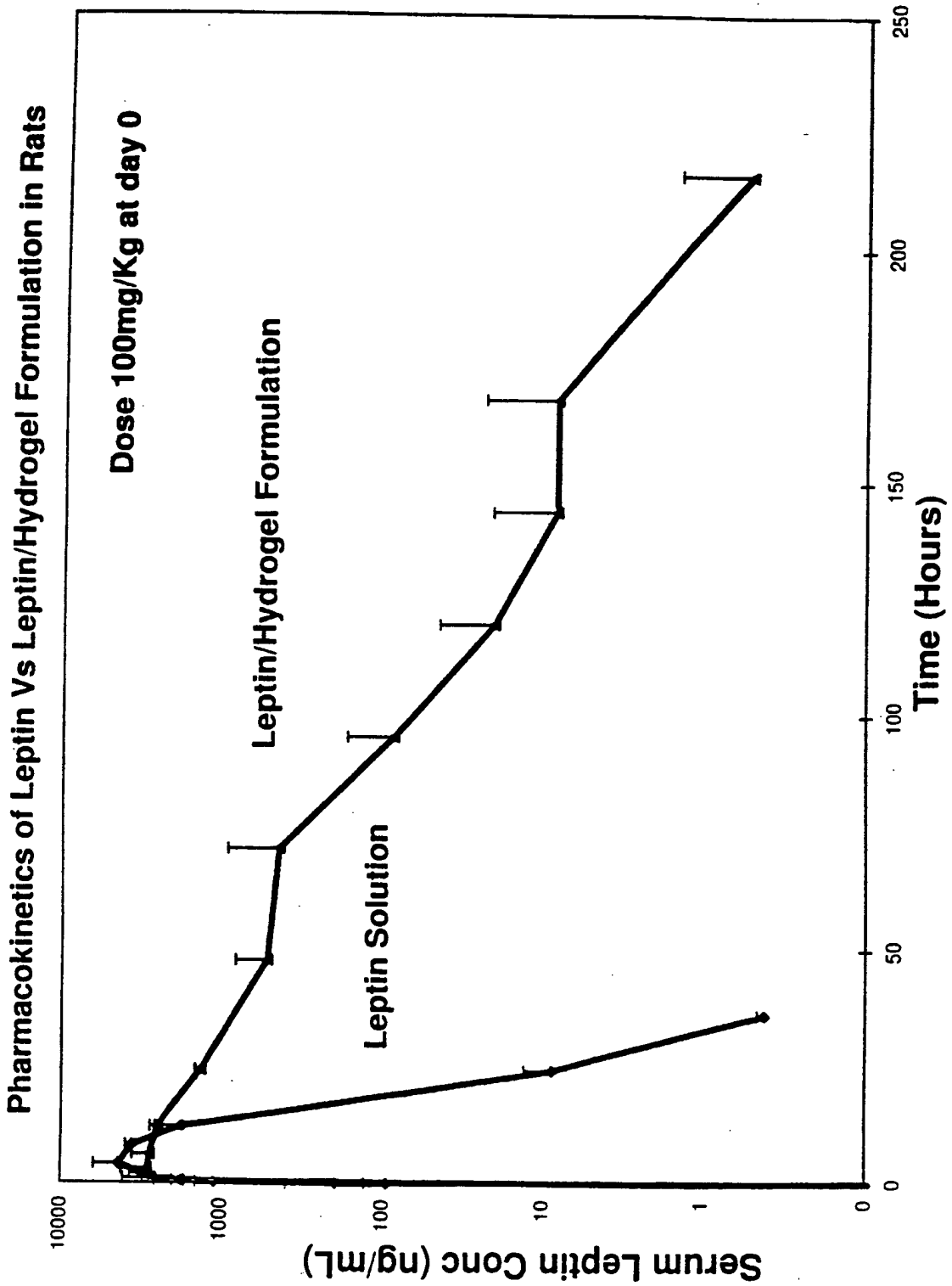

Pharmacokinetics of Leptin Vs Leptin/Hydrogel Formulation in Rats
Dose 100mg/Kg at day 0
Leptin/Hydrogel Formulation
Leptin Solution
Serum Leptin Conc (ng/mL)
10000
1000
100
10
1
0
Time (Hours)
0
50
100
150
200
250

FIG. 6

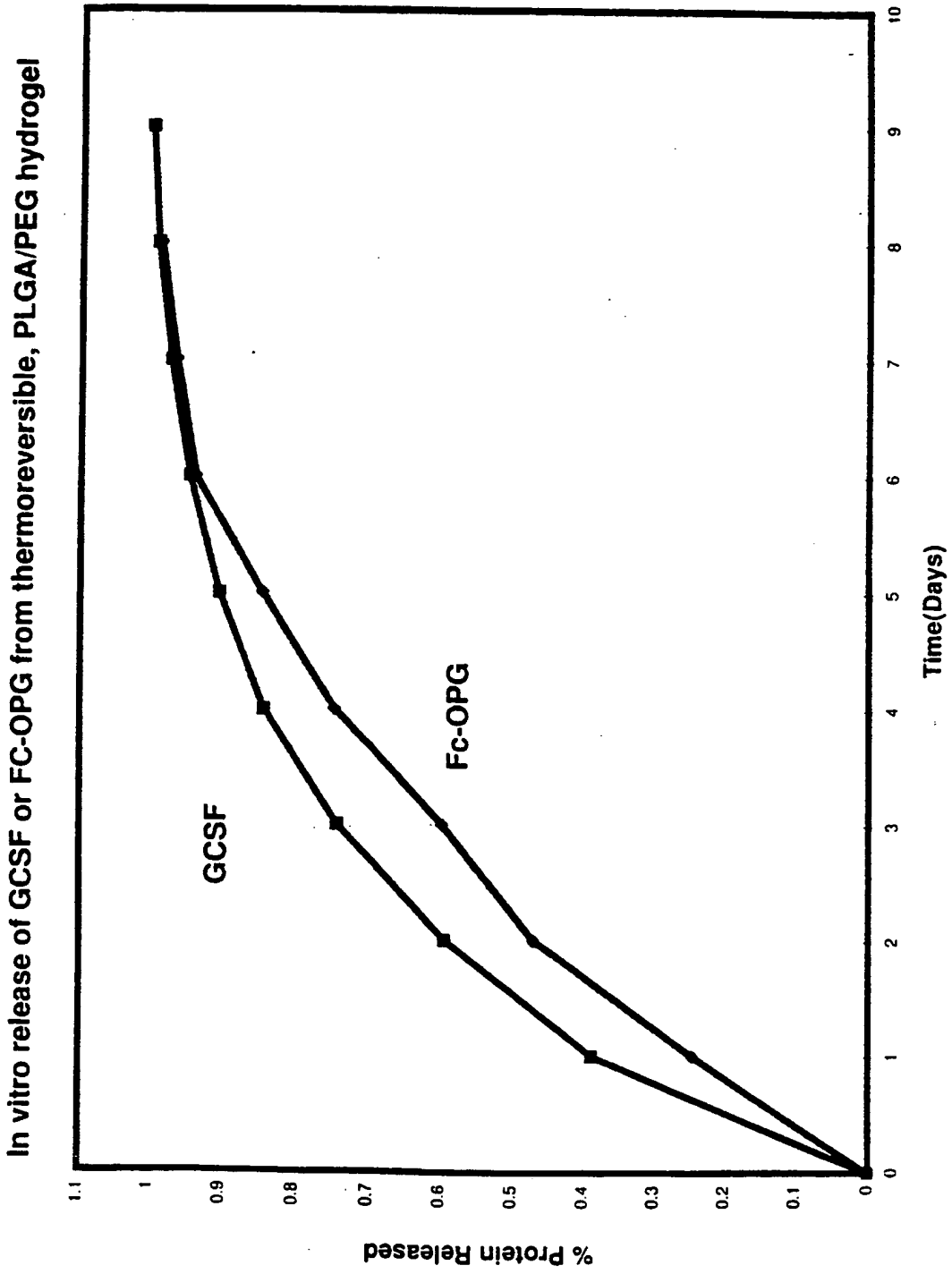

In vitro release of GCSF or FC-OPG from thermoreversible, PLGA/PEG hydrogel
% Protein Released
Time(Days)
GCSF
Fc-OPG
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
1.1
0
1
2
3
4
5
6
7
8
9
10

FIG. 7

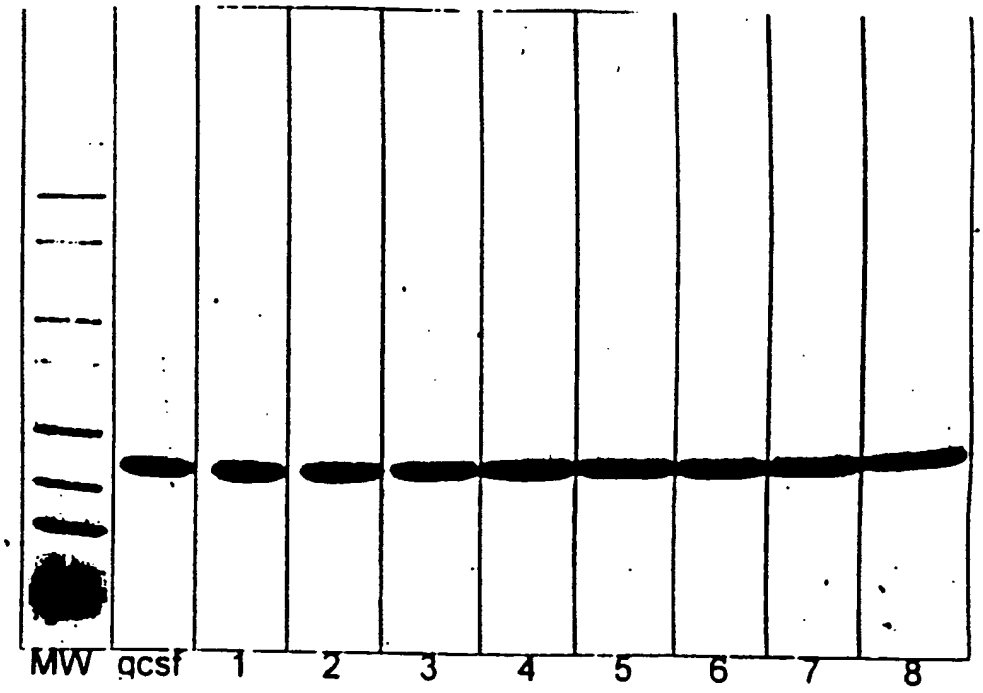
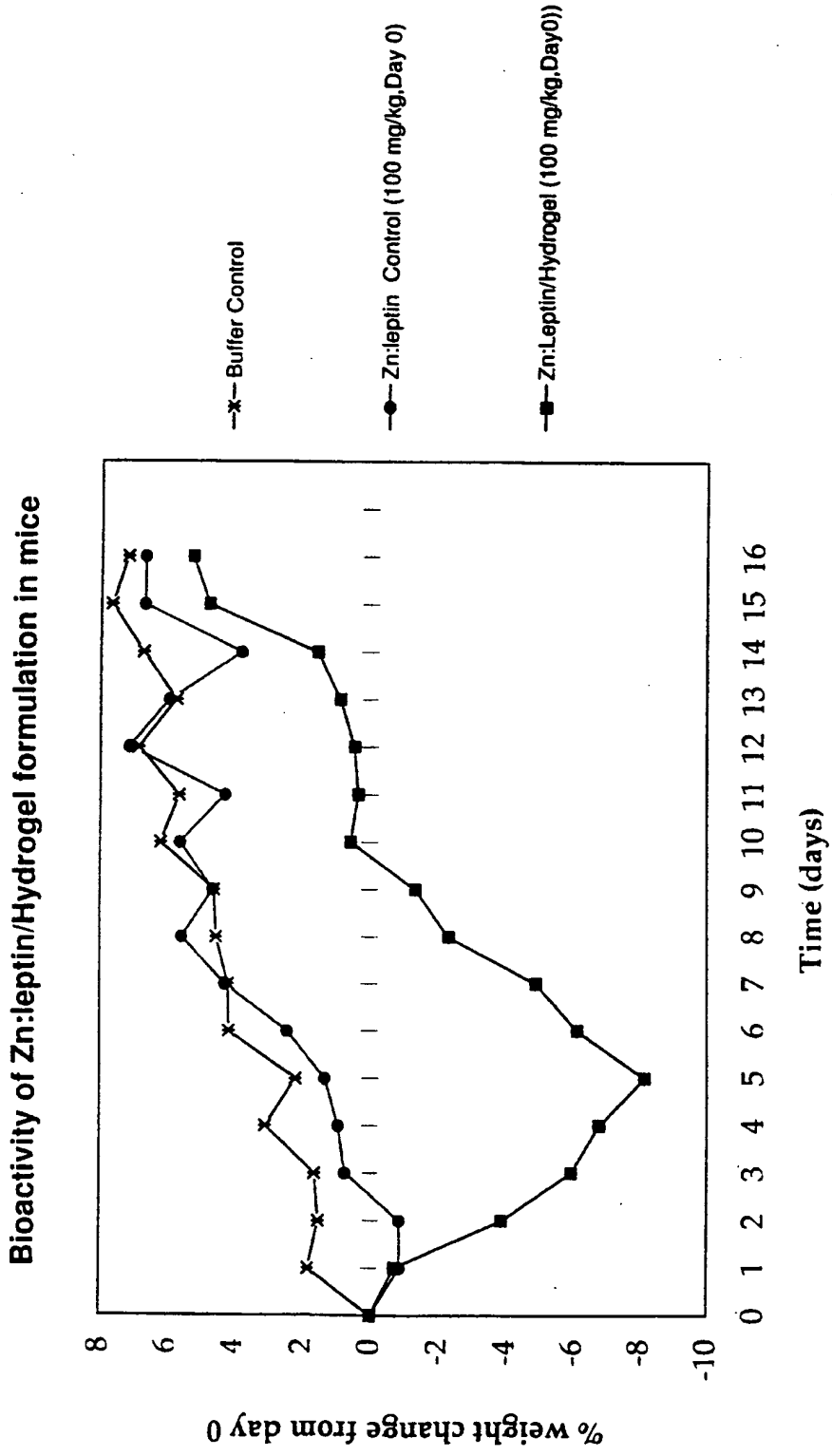


FIG. 8


Bioactivity of Zn:leptin/Hydrogel formulation in mice
Buffer Control
Zn:leptin Control (100 mg/kg,Day 0)
Zn:Leptin/Hydrogel (100 mg/kg,Day0))
% weight change from day 0
8
6
4
2
0
-2
-4
-6
-8
-10
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Time (days)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/14206

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K47/34

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 99 18142 A (MACROMED, INC.) 15 April 1999 (1999-04-15) the whole document	1-19
X	WO 97 15287 A (MACROMED, INC.) 1 May 1997 (1997-05-01) & US 5 702 717 A cited in the application	1,9-11, 18,19

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 November 1999	15/11/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Benz, K

1

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

ernational application No.
PCT/US 99/ 14206

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 11-19 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

nformation on patent family members

Inte··ational Application No
PC·/US 99/14206

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9918142 A	15-04-1999	AU 9678098 A	27-04-1999
WO 9715287 A	01-05-1997	US 5702717 A	30-12-1997
		AU 7520096 A	15-05-1997
		EP 0863745 A	16-09-1998

CORRECTED VERSION*

PCT

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International Bureau

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A1

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(30) Priority Data:
09/107,334 30 June 1998 (30.06.98) US

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(72) Inventor: SHAH, Subodh; 1890 West Hillcrest Drive #380, Newbury Park, CA 91320 (US).

(74) Agents: ODRE, Steven, M. et al.; Amgen Inc., One Amgen Center Drive, Thousand Oaks, CA 91320–1799 (US).

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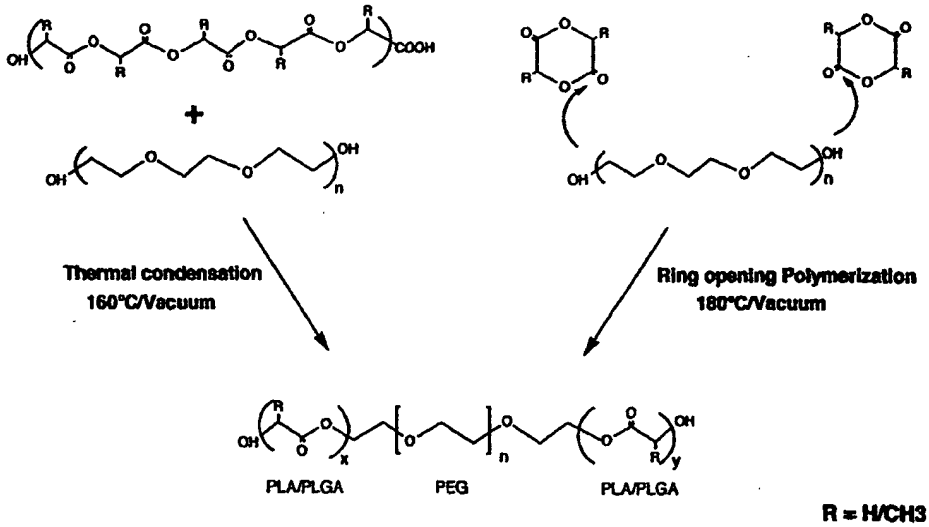
Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: THERMOSENSITIVE BIODEGRADABLE HYDROGELS FOR SUSTAINED DELIVERY OF BIOLOGICALLY ACTIVE AGENTS



(A-B-A) BLOCK COPOLYMER

(57) Abstract

The present invention relates generally to the development of pharmaceutical compositions which provide for sustained release of biologically active polypeptides. More specifically, the invention relates to the use of thermosensitive, biodegradable hydrogels, consisting of a block copolymer of poly(d, l- or l-lactic acid) (PLA) or poly(lactide-co-glycolide) (PLGA) and polyethylene glycol (PEG), for the sustained delivery of biologically active agents.

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THERMOSENSITIVE BIODEGRADABLE HYDROGELS FOR SUSTAINED DELIVERY OF BIOLOGICALLY ACTIVE AGENTS

FIELD OF THE INVENTION

The present invention relates to the use of thermosensitive, biodegradable hydrogels, consisting of a block copolymer of poly(d,l- or l-lactic acid)(PLA) or poly(lactide-co-glycolide)(PLGA) and polyethylene glycol (PEG), for the sustained delivery of biologically active agents.

BACKGROUND OF THE INVENTION

Due to recent advances in genetic and cell engineering technologies, proteins known to exhibit various pharmacological actions *in vivo* are capable of production in large amounts for pharmaceutical applications. Such proteins include erythropoietin (EPO), granulocyte colony-stimulating factor (G-CSF), interferons (alpha, beta, gamma, consensus), tumor necrosis factor binding protein (TNFbp), interleukin-1 receptor antagonist (IL-1ra), brain-derived neurotrophic factor (BDNF), kerantinocyte growth factor (KGF), stem cell factor (SCF), megakaryocyte growth differentiation factor (MGDF), osteoprotegerin (OPG), glial cell line derived neurotrophic factor (GDNF) and obesity protein (OB protein). OB protein may also be referred to herein as leptin.

Because proteins such as leptin generally have short *in vivo* half-lives and negligible oral bioavailability, they are typically administered by frequent injection, thus posing a significant physical burden on the patient (e.g., injection site reactions are particularly problematic with many leptin

formulations) and associated administrative costs. As such, there is currently a great deal of interest in developing and evaluating sustained-release formulations. Effective sustained-release formulations can provide a means of controlling blood levels of the active ingredient, and also provide greater efficacy, safety, patient convenience and patient compliance. Unfortunately, the instability of most proteins (e.g. denaturation and loss of bioactivity upon exposure to heat, organic solvents, etc.) has greatly limited the development and evaluation of sustained-release formulations.

Biodegradable polymer matrices have thus been evaluated as sustained-release delivery systems. Attempts to develop sustained-release formulations have included the use of a variety of biodegradable and non-biodegradable polymer (e.g. poly(lactide-co-glycolide)) microparticles containing the active ingredient (see e.g., Wise et al., *Contraception*, 8:227-234 (1973); and Hutchinson et al., *Biochem. Soc. Trans.*, 13:520-523 (1985)), and a variety of techniques are known by which active agents, e.g. proteins, can be incorporated into polymeric microspheres (see e.g., U.S. Patent No. 4,675,189 and references cited therein).

Utilization of the inherent biodegradability of these materials to control the release of the active agent and provide a more consistent sustained level of medication provides improvements in the sustained release of active agents. Unfortunately, some of the sustained release devices utilizing microparticles still suffer from such things as: active agent aggregation formation; high initial bursts of active agent with minimal release thereafter; and incomplete release of active agent.

Other drug-loaded polymeric devices have also been investigated for long term, therapeutic treatment of various diseases, again with much attention being directed to polymers derived from alpha hydroxycarboxylic acids, especially lactic acid in both its racemic and optically active form, and glycolic acid, and copolymers thereof. These polymers are commercially available and have been utilized in FDA-approved systems, e.g., the Lupron Depot™, which consists of injectable microcapsules which release leuprolide acetate for about 30 days for the treatment of prostate cancer.

Various problems identified with the use of such polymers include: inability of certain macromolecules to diffuse out through the matrix; deterioration and decomposition of the drug (e.g., denaturation caused by the use of organic solvents); irritation to the organism (e.g. side effects due to use of organic solvents); low biodegradability (such as that which occurs with polycondensation of a polymer with a multifunctional alcohol or multifunctional carboxylic acid, i.e., ointments); and slow rates of degradation.

The use of polymers which exhibit reverse thermal gelation have also been reported. For example, Okada et al., Japanese Patent Application 2-78629 (1990) describe biodegradable block copolymers synthesized by transesterification of poly(lactic acid)(PLA) or poly(lactic acid)/glycolic acid (PLA/GA) and poly(ethylene glycol)(PEG). PEGs with molecular weights ranging from 200 to 2000, and PLA/GA with molecular weights ranging from 400 to 5000 were utilized. The resultant product was miscible with water and formed a hydrogel. The Okada et al.

reference fails to provide any demonstration of sustained delivery of drugs using the hydrogels.

Cha et al., U.S. Patent No. 5,702,717 (Dec. 30, 1997) describe systems for parenteral delivery of a drug comprising an injectable biodegradable block copolymeric drug delivery liquid having reverse thermal gelation properties, i.e., ability to form semi-solid gel, emulsions or suspension at certain temperatures. Specifically, these thermosensitive gels exist as a mobile viscous liquid at low temperatures, but form a rigid semisolid gel at higher temperatures. Thus, it is possible to use these polymers to design a formulation which is liquid at room temperature or at lower temperature and below, but gels once injected, thus producing a depot of drug at the injection site. The systems described by Cha et al. utilize a hydrophobic A polymer block comprising a member selected from the group consisting of poly(α-hydroxy acids) and poly(ethylene carbonates) and a hydrophilic B polymer block comprising a PEG. The Cha et al. system requires that less than 50% by weight hydrophobic A polymer block be utilized and greater than 50% by weight hydrophilic B polymer block be utilized. Interestingly, however, it appears that several of the disclosed hydrogels might not be commercially useful in that the lower critical solution temperature (LCST) for many of the gels is greater than 37°C. Although Cha et al. propose use of their hydrogels for controlled release of drugs, no such demonstration is provided.

Martini et al., *J. Chem. Soc.*, 90(13):1961-1966 (1994) describe low molecular weight ABA type triblock copolymers which utilize hydrophobic poly(ε-caprolactone)(PCL) and PEG. Unfortunately, *in*

vitro degradation rates for these copolymers was very slow, thus calling into question their ability as sustained-release systems.

Stratton et al., PCT/US97/13479 (WO 98/02142) January 22, 1998, describe pharmaceutical compositions comprising a polymeric matrix having thermal gelation properties, for the delivery of proteins. The class of block copolymers described are generically referred to as polyoxyethylene-polyoxypropylene condensates (also known as Pluronics). Unfortunately, systems utilizing Pluronics suffer from the fact that they are toxic to body organs and are nonbiodegradable. Moreover, only high molecular weight Pluronics at higher concentrations (25-40 wt.%) exhibit thermoreversible gelation.

It is thus the object of the present invention to provide thermosensitive, biodegradable hydrogels for the sustained delivery of drugs. The hydrogels of the present invention utilize copolymer compositions which provide for instant gelation, and which possess the necessary rate of degradation to make use of the hydrogels commercially practical.

SUMMARY OF THE INVENTION

In one embodiment, the present invention provides pharmaceutical compositions comprising an effective amount of a biologically active agent incorporated into a polymeric matrix, said polymeric matrix comprising a block copolymer which is biodegradable, exhibits thermal gelation behavior, and is capable of providing for the sustained-release of the biologically active agent.

In another embodiment, the present invention provides a method for the parenteral administration of

a biologically active agent in a biodegradable polymeric matrix to a warm blooded animal, wherein a gel depot is formed within the body of said animal and the biologically active agent is released from the depot at a controlled rate concomitant with biodegradation of the polymeric matrix.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the two methods by which the A-B-A block copolymers of the present invention can be prepared.

Figure 2 depicts the *in vitro* release characteristics of leptin released from a hydrogel (PLGA/PEG (74%/26% w/w)). % protein released is plotted vs. time (days).

Figure 3 is a photograph of an SDS-PAGE gel characterizing samples of leptin released from a hydrogel on various days. Lane 1 is a leptin standard; Lane 2 and 15 contain molecular weight markers; and Lanes 3-14 represent leptin samples at day 1-12, respectively.

Figure 4 depicts the *in vivo* bioactivity for various leptin-containing hydrogel (PLGA/PEG (74%/26% w/w)) formulations. The -✱- depicts a 20mM acetate, pH 4.8, buffer control, 100µl on day 0; -●- depicts a hydrogel (74%/26%) control, 100µl on day 0; -◆- depicts leptin (20 mg/mL), 100 mg/kg, 100µl on day 0; -Δ- depicts leptin (2 mg/mL), 10 mg/kg, 100µl daily; -▲- depicts a leptin-containing hydrogel (PLGA/PEG (74%/26% w/w)), 20 mg/mL leptin, 100 mg/kg, 100µl on

day 0; and -■- depicts a leptin-containing hydrogel (PLGA/PEG (74%/26% w/w)), 20 mg/mL leptin, 200 mg/kg, 200µl on day 0. % body weight change (from the day 0 body weight) is plotted vs. time (days).

Figure 5 depicts the pharmacokinetics for a leptin-containing hydrogel (PLGA/PEG (74%/26% w/w)) (-▲-) and leptin solution (-◆-). Serum leptin concentration (ng/mL) is plotted vs. time (hours).

Figure 6 depicts the *in vitro* release characteristics for GCSF from a GCSF-containing hydrogel (PLGA/PEG (74%/26% w/w)) (-◆-) and Fc-OPG from a Fc-OPG-containing hydrogel (PLGA/PEG (74%/26% w/w)) (-■-). % protein released is plotted vs. time (days).

Figure 7 is a photograph of an SDS-PAGE gel characterizing samples of GCSF released from a hydrogel on various days. Lane 1 is a leptin standard; Lane 2 and 15 contain molecular weight markers; and Lanes 3-14 represent leptin samples at day 1-12, respectively.

Figure 8 depicts the *in vivo* bioactivity for a Zn:leptin-containing hydrogel (PLGA/PEG (74%/26% w/w)) formulation. -✱- depicts a 20mM acetate, pH 4.8, buffer control, 100µl on day 0; -•- depicts Zn:leptin, 100 mg/kg, 100µl on day 0; and -■- depicts a Zn:leptin-containing hydrogel (PLGA/PEG (74%/26% w/w)), 20 mg/mL leptin, 100 mg/kg, 100µl on day 0. % body weight change (from the day 0 body weight) is plotted vs. time (days).

DETAILED DESCRIPTION OF THE INVENTION

As used herein, the following terms shall have the following meaning:

"Reverse thermal gelation" is defined as meaning the temperature below which a copolymer is soluble in water and above which the block copolymer forms a semi-solid, i.e. gels, emulsions, dispersions and suspensions.

"LCST", or lower critical solution temperature, is defined as meaning the temperature at which a biodegradable block copolymer undergoes reverse thermal gelation. For purposes of the present invention, the term "LSCT" can be used interchangeably with "reverse thermal gelation temperature".

"Depot" is defined as meaning a drug delivery liquid which, following injection into a warm blooded animal, has formed a gel upon having the temperature raised to or above the LCST.

"Biodegradable" is defined as meaning that the block copolymer will erode or degrade *in vivo* to form smaller non-toxic components.

"Parenteral administration" is defined as meaning any route of administration other than the alimentary canal, including, for example, subcutaneous and intramuscular.

The present invention involves utilization of block copolymers having hydrophobic ("A") block segments and hydrophilic ("B") block segments. The block copolymers are triblock copolymers, e.g., ABA or BAB type block copolymers, which possess reverse thermal gelation properties and are biodegradable and biocompatible. Importantly, triblock copolymers of the present invention provide instant gelation and possess the necessary rate of degradation to be commercially useful.

Biodegradable hydrophobic A block segments contemplated for use include poly(α-hydroxy acid) members derived from or selected from the group consisting of homopolymers and copolymers of poly(lactide)s (d,l- or l- forms), poly(glycolide)s, polyanhydrides, polyorthoesters, polyetheresters, polycaprolactone, polyesteramides, polycarbonate, polycyanoacrylate, polyurethanes, polyacrylate, blends and copolymers thereof.

The term "PLGA" as used herein is intended to refer to a polymer of lactic acid alone, a polymer of glycolic acid alone, a mixture of such polymers, a copolymer of glycolic acid and lactic acid, a mixture of such copolymers, or a mixture of such polymers and copolymers. Preferably, the biodegradable A block polymer will be poly lactide-co-glycolide (PLGA), and the PLGA composition will be such that the necessary rate of gelation and rate of degradation are obtained.

The range of molecular weights contemplated for the polymers to be used in the present processes can be readily determined by a person skilled in the art based upon such factors the desired polymer degradation rate. Typically, the range of molecular weight for the A block will be 1000 to 20,000 Daltons.

Hydrophilic B block segments contemplated for use include polyethylene glycols having average molecular weights of between about 500 and 10,000.

The copolymer compositions for the block copolymers of the present invention are specially regulated to assure retention of the desired water-solubility and gelling properties, i.e., the ratios must be such that the block copolymers possess water solubility at temperatures below the LCST, and such that there is instant gelation under physiological conditions (i.e. pH 7.0 and 37°C) so as to minimize the

initial burst of drug. In the hydrogels of the present invention the hydrophobic A block makes up 55% to 90% by weight of the copolymer and the hydrophilic B block makes up 10% to 45% of the copolymer.

The concentration at which the block copolymers of the present invention remain soluble below the LCST are generally up to about 60% by weight, with 10%-30% preferred. The concentration utilized will depend upon the copolymer composition actually used, as well as whether or not a gel or emulsion is desired.

The thermosensitive block copolymers of the present invention can be prepared by thermal condensation. In a typical experiment, A-B-A block copolymers of PLGA/PLA (block A) and PEG (block B) are synthesized by mixing either homopolymer of poly lactide (PLA) or copolymer of poly lactide-co-gycolide (PLGA) with polyethylene glycol (PEG) and allowing di-hydroxy PEG to react with PLGA or PLA at 160°C under reduced pressure. Different weight ratios of PLGA and PEG were used for thermal condensation to obtain a series of block copolymers with desirable copolymer composition and block lengths. Copolymer composition and relative block lengths were confirmed by ^{1}H-NMR spectroscopy.

Alternatively, the copolymers could be synthesized in a melt process which involves ring opening polymerization of A block using B block as the initiator. In a typical experiment, the ABA triblock copolymer is prepared by stannous octoate catalyzed ring-opening polymerization of d,l-dilactide (or PLGA) using α,ω-dihydroxy-terminated PEG as the initiator. The mole ratio of B block to d,l-dilactide (or PLGA) is used to control the lengths of the A blocks, and provide a series of polymers with increasing A block

contents and hydrophobicites. The relative A and B block lengths can be confirmed by ^{1}H-NMR spectroscopy.

The process used to mix the copolymers with a biologically active agent and/or other materials involves dissolving the ABA block copolymers in an aqueous solution, followed by addition of the biologically active agent (in solution, suspension or powder), followed by thorough mixing to assure a homogeneous mixing of the biologically active agent throughout the copolymer. Alternatively, the process can involve the dissolving of the ABA block copolymer in a biologically active agent-containing solution. In either case, the process is conducted at a temperature lower than the gelation temperature of the copolymer and the material is implanted into the body as a solution which then gels or solidifies into a depot in the body. In the compositions of the present invention, the biologically active agent will generally have a concentration in the range of 0 to 200 mg/mL.

Buffers contemplated for use in the preparation of the biologically active agent-containing hydrogels are buffers which are all well known by those of ordinary skill in the art and include sodium acetate, Tris, sodium phosphate, MOPS, PIPES, MES and potassium phosphate, in the range of 25mM to 500mM and in the pH range of 4.0 to 8.5.

It is also envisioned that other excipients, e.g., various sugars, salts, or surfactants, may be included in the biologically active agent-containing hydrogels of the present invention in order to alter the LCST or rate of gelation of the gels. The ability to alter the rate of gelation and/or LCST is important and an otherwise non-useful hydrogel may be made useful by addition of such excipients. Examples of such sugars include glucose or sucrose in the range of 5% to

20%. Examples of such salts include sodium chloride or zinc chloride in the range of 0.5% to 10%.

As used herein, biologically active agents refers to recombinant or naturally occurring proteins, whether human or animal, useful for prophylactic, therapeutic or diagnostic application. The biologically active agent can be natural, synthetic, semi-synthetic or derivatives thereof. In addition, biologically active agents of the present invention can be perceptible. A wide range of biologically active agents are contemplated. These include but are not limited to hormones, cytokines, hematopoietic factors, growth factors, antiobesity factors, trophic factors, anti-inflammatory factors, small molecules and enzymes (see also U.S. Patent No. 4,695,463 for additional examples of useful biologically active agents). One skilled in the art will readily be able to adapt a desired biologically active agent to the compositions of present invention.

Proteins contemplated for use would include but are not limited to interferon consensus (see, U.S. Patent Nos. 5,372,808, 5,541,293 4,897,471, and 4,695,623 hereby incorporated by reference including drawings), interleukins (see, U.S. Patent No. 5,075,222, hereby incorporated by reference including drawings), erythropoietins (see, U.S. Patent Nos. 4,703,008, 5,441,868, 5,618,698 5,547,933, and 5,621,080 hereby incorporated by reference including drawings), granulocyte-colony stimulating factors (see, U.S. Patent Nos. 4,810,643, 4,999,291, 5,581,476, 5,582,823, and PCT Publication No. 94/17185, hereby incorporated by reference including drawings), stem cell factor (PCT Publication Nos. 91/05795, 92/17505 and 95/17206, hereby incorporated by reference including drawings), and leptin (OB protein) (see

PCT publication Nos. 96/40912, 96/05309, 97/00128, 97/01010 and 97/06816 hereby incorporated by reference including figures).

It is desirable to have such protein containing sustained-release compositions as such compositions could serve to enhance the effectiveness of either exogenously administered or endogenous protein, or could be used, for example, to reduce or eliminate the need for exogenous protein administration.

Moreover, because the materials utilized in the present invention are biocompatible and biodegradable, use of the protein compositions of the present invention help prevent adverse injection site reactions sometimes associated with i.v. injections of various proteins such as leptin.

In addition, biologically active agents can also include insulin, gastrin, prolactin, adrenocorticotropic hormone (ACTH), thyroid stimulating hormone (TSH), luteinizing hormone (LH), follicle stimulating hormone (FSH), human chorionic gonadotropin (HCG), motilin, interferons (alpha, beta, gamma), tumor necrosis factor (TNF), tumor necrosis factor-binding protein (TNF-bp), interleukin-1 receptor antagonist (IL-1ra), brain derived neurotrophic factor (BDNF), glial derived neurotrophic factor (GDNF), neurotrophic factor 3 (NT3), fibroblast growth factors (FGF), neurotrophic growth factor (NGF), bone growth factors such as osteoprotegerin (OPG), insulin-like growth factors (IGFs), macrophage colony stimulating factor (M-CSF), granulocyte macrophage colony stimulating factor (GM-CSF), megakaryocyte derived growth factor (MGDF), keratinocyte growth factor (KGF), thrombopoietin, platelet-derived growth factor (PGDF), colony simulating growth factors (CSFs), bone

morphogenetic protein (BMP), superoxide dismutase (SOD), tissue plasminogen activator (TPA), urokinase, streptokinase and kallikrein. The term proteins, as used herein, includes peptides, polypeptides, consensus molecules, analogs, derivatives or combinations thereof.

Also included are those polypeptides with amino acid substitutions which are "conservative" according to acidity, charge, hydrophobicity, polarity, size or any other characteristic known to those skilled in the art. *See generally*, Creighton, Proteins, W.H. Freeman and Company, N.Y., (1984) 498 pp. plus index, *passim*. One may make changes in selected amino acids so long as such changes preserve the overall folding or activity of the protein. Small amino terminal extensions, such as an amino-terminal methionine residue, a small linker peptide of up to about 20-25 residues, or a small extension that facilitates purification, such as a poly-histidine tract, an antigenic epitope or a binding domain, may also be present. *See*, in general, Ford et al., *Protein Expression and Purification* 2:95-107 (1991), which is herein incorporated by reference. Polypeptides or analogs thereof may also contain one or more amino acid analogs, such as peptidomimetics.

In general, comprehended by the invention are pharmaceutical compositions comprising effective amounts of chemically modified protein, or derivative products, together with pharmaceutically acceptable diluents, preservatives, solubilizers, emulsifiers, adjuvants and/or carriers needed for administration. (See PCT 97/01331 hereby incorporated by reference.) The optimal pharmaceutical formulation for a desired biologically active agent will be determined by one

skilled in the art depending upon the route of administration and desired dosage. Exemplary pharmaceutical compositions are disclosed in Remington's Pharmaceutical Sciences (Mack Publishing Co., 18th Ed., Easton, PA, pgs. 1435-1712 (1990)).

The pharmaceutical compositions of the present invention are administered as a liquid via intramuscular or subcutaneous route and undergo a phase change wherein a gel is formed within the body, since the body temperature will be above the gelation temperature of the material. The release rates and duration for the particular biologically active agents will be a function of, *inter alia*, hydrogel density and the molecular weight of the agent.

Therapeutic uses of the compositions of the present invention depend on the biologically active agent used. One skilled in the art will readily be able to adapt a desired biologically active agent to the present invention for its intended therapeutic uses. Therapeutic uses for such agents are set forth in greater detail in the following publications hereby incorporated by reference including drawings. Therapeutic uses include but are not limited to uses for proteins like interferons (*see*, U.S. Patent Nos. 5,372,808, 5,541,293, hereby incorporated by reference including drawings), interleukins (*see*, U.S. Patent No. 5,075,222, hereby incorporated by reference including drawings), erythropoietins (*see*, U.S. Patent Nos. 4,703,008, 5,441,868, 5,618,698 5,547,933, and 5,621,080 hereby incorporated by reference including drawings), granulocyte-colony stimulating factors (*see*, U.S. Patent Nos. 4,999,291, 5,581,476, 5,582,823, 4,810,643 and PCT Publication No. 94/17185, hereby incorporated by reference including drawings), stem cell factor (PCT Publication Nos. 91/05795, 92/17505

and 95/17206, hereby incorporated by reference including drawings), and the OB protein (*see* PCT publication Nos. 96/40912, 96/05309, 97/00128, 97/01010 and 97/06816 hereby incorporated by reference including figures). In addition, the present compositions may also be used for manufacture of one or more medicaments for treatment or amelioration of the conditions the biologically active agent is intended to treat.

In the sustained-release compositions of the present invention, an effective amount of active ingredient will be utilized. As used herein, sustained release refers to the gradual release of active ingredient from the polymer matrix, over an extended period of time. The sustained release can be continuous or discontinuous, linear or non-linear, and this can be accomplished using one or more polymer compositions, drug loadings, selection of excipients, or other modifications. The sustained release will result in biologically effective serum levels of the active agent (typically above endogenous levels) for a period of time longer than that observed with direct administration of the active agent. Typically, a sustained release of the active agent will be for a period of a week or more, preferably up to one month.

The following examples are offered to more fully illustrate the invention, but are not to be construed as limiting the scope thereof.

Materials

Low molecular weight (Mn 2000-6000) PLGA (poly Lactic acid-co-Glycolic acid) and PLA (poly Lactic acid) were synthesized by direct thermal condensation of glycolic acid and lactic acid at 180°C under reduced pressure. High molecular weight PLGAs were obtained from B.I. Chemicals. Polyethylene glycols (PEG) were obtained from Fluka Chemicals. Leptin, zinc-leptin, GCSF, Fc-Leptin, and Fc-OPG were obtained from Amgen Inc. All other chemicals are from sources well known in the art.

Example 1

This example describes synthesis of a PLGA/PEG, A-B-A (PLGA-PEG-PLGA), block copolymer by thermal condensation. The thermal condensation method is generally depicted in Figure 1.

30 g PLGA (75%/25% LA/GA ratio) (Mn 3740, MW 7050) and 10.7 g polyethylene glycol (MW 1000) were placed into a three-neck round bottom flask equipped with a thermometer, a nitrogen gas inlet, and a distillation condenser connected to a vacuum pump. After addition of the polymers, the temperature of the reaction mixture was raised slowly to 160°C under nitrogen purging. The condensation reaction was further carried out at 160°C for 14 hours under 500 millitorr pressure and with continuous bubbling of dry nitrogen gas. At the end of the condensation reaction, the reaction mixture was cooled, dissolved in methylene chloride and precipitated with an excess of cold isopropanol.

The isolated polymer was dried at 40°C under vacuum for 48 hours. The molecular weight of the block copolymer was determined by gel permeation chromatography (GPC) using polystyrene standards. The copolymer

composition and relative block lengths were determined by ^{1}H-NMR.

The PLGA/PEG block copolymer dissolved either in 100mM sodium acetate, pH 6.0, or 100mM sodium phosphate, pH 7.0, exhibited a unique thermoreversible property (solution below room temperature and gel above room temperature, sol-gel-sol) with lower critical solution temperature (LCST) at about 30°C to 35°C.

Example 2

This example describes the synthesis of PLGA/PEG, A-B-A (PLGA-PEG-PLGA), block copolymers using PLGA with different lactic acid to glycolic acid ratios.

The synthesis and characterization procedures described in Example 1 were utilized to prepare PLGA/PEG block copolymers using PLGA with different LA to GA ratios (see Table 1 below). The block copolymers listed below showed thermoreversibility (Sol-gel-sol) with LCST at about 30°C to 35°C.

Table 1

PLGA (LA/GA ratio) wt (g)	PEG 1000 wt (g)	PLGA/PEG wt ratio	PLGA/PEG calculated	Molar ratio by NMR
PLA (100%) 45g (Mn 3480, MW 6590)	17.55g	72/28	1.56	1.48
PLGA (75/25%) 30g (Mn 3740, MW 7090)	11.74g	72/28	1.65	1.56
PLGA (50/50%) 30g (Mn 3480/MW 6590)	10.71g	74/26	1.8	1.78
PLGA (56/44%) 40g (Mn 3480/MW 6590)	15.60g	72/28	1.71	1.66

Example 3

This example describes the synthesis of PLGA/PEG, A-B-A (PLGA-PEG-PLGA), block copolymers using different weight ratios of PLGA and PEG.

The synthesis and characterization procedure described in Example 1 were utilized to prepare PLGA/PEG block copolymers with various PLGA to PEG ratios (see Table 2 below). All of the block copolymers listed below showed thermoreversibility (sol-gel-sol) with LCST in the range of 25°C - 35°C.

Table 2

PLGA (75/25) Mn 3740,MW 7090	PEG 1000 wt (g)	PLGA/PEG wt ratio	PLGA/PEG calculated	Molar ratio by NMR
30g	9.47g	76/24	2.03	
40g	14.28g	74/26	1.79	1.70
40g	14.90g	73/27	1.72	1.65
30g	11.84g	72/28	1.65	1.56
30g	12.63g	70/30	1.53	1.50
30g	14.21g	68/32	1.36	1.32
30g	15.48g	66/34	1.24	1.17
30g	16.70g	64/36	1.15	1.08
30g	18.40g	62/38	1.05	

Example 4

This example describes the preparation of a leptin/hydrogel formulation and the methods used to determine the *in vitro* release kinetics, *in vivo* release kinetics, and pharmacokinetics of the leptin/hydrogel.

Preparation of leptin/hydrogel formulation

The PLGA/PEG block copolymer described in Example 1 was dissolved in 50mM sodium acetate, pH 6.0. Leptin solution (formulated in 20mM acetate, pH 4.8) was slowly added to the hydrogel solution and the mixture was gently swirled on an orbital shaker at 5°C to assure a homogeneous mixing of leptin throughout the hydrogel solution. The final concentration of the copolymer in the final leptin/hydrogel formulation was 10-50% (w/w) and the leptin concentration was in the range of 0-100 mg/ml. The final leptin/hydrogel formulation was filtered through 0.2μ filter and stored either as a solution at 5°C or stored as a frozen mass at -20°C.

Alternatively, the leptin/hydrogel formulation was prepared by dissolving the PLGA/PEG block copolymer in a leptin solution. The leptin solution concentration was varied to obtain desirable copolymer as well as the desired protein concentration in final formulation.

In vitro Release Study

The *in vitro* release of leptin from the leptin/hydrogel was carried out in 20mM sodium phosphate, 5% sorbitol, pH 7.4, at 37°C. 1 ml of leptin/hydrogel solution formulation was placed in a glass vial at 37°C. Upon gelation of the leptin/hydrogel formulation, 1 ml of 20mM phosphate, 5% sorbitol, pH 7.4, buffer was added directly above and in contact with the gel. The amount of leptin released in the top buffer phase was determined by UV spectrophotometer at 280nm as well as by SEC-HPLC at 220nm. To maintain a perfect sink condition the aqueous receptor phase above the gel was completely

removed at definite time intervals and replaced by fresh buffer. The % leptin released over time is depicted in Figure 2. The integrity of the leptin released from the hydrogel formulation was confirmed by HPLC (data not shown) and gel electrophoresis (SDS-PAGE)(see Figure 3).

***In vivo* bioactivity**

The *in vivo* bioactivity of leptin/hydrogel formulations were evaluated in normal mice. Mice were injected subcutaneously (s.c.) with either: a) 0.1 ml of 20mM acetate buffer, pH 4.8, (n=5, day 0 only); (b) 0.1 ml of 20 mg/ml leptin formulated in 20mM acetate buffer, pH 4.8 (n=5, 100 mg/kg, day 0 only); (c) 0.1 ml of 2 mg/ml leptin formulated in 20mM acetate buffer, pH 4.8 (n=5, 10 mg/kg, daily); (d) 0.1 ml of a leptin/hydrogel (74/26% (PLGA/PEG)(w/w)) formulation consisting of 20 mg/ml leptin, in 20mM acetate, pH 4.8 (n=5, 100 mg/kg, day 0 only); (e) 0.2 ml of a leptin/hydrogel (74/26% (PLGA/PEG)(w/w)) formulation consisting of 20 mg/ml leptin, in 20mM acetate, pH 4.8 (n=5, 200 mg/kg ,day 0 only); or (f) 0.1 ml of a hydrogel (74/26% (PLGA/PEG)(w/w)) control, formulated in 50mM acetate, pH 6.0 (n=5, day 0 only).

% body weight change (from the day 0 body weight) was determined by weighing the animals daily until the body weight of the animals injected with sample (b), (d) and (e) reached the body weights of the animals injected with buffer control (sample (a)). Importantly, a single s.c. injection of 100 mg/kg leptin/hydrogel formulation (sample (d)) showed sustained weight loss in normal mice over a 10 day period. The duration of sustained weight loss effect was further extended up to 14 days when the dose was

increased to 200 mg/kg (sample (e)). It was also observed that a single injection of 100 mg/kg or 200 mg/kg leptin/hydrogel at day '0' was more efficacious up to 14 days than daily injections of 10 mg/kg leptin without hydrogel. These results are depicted in Figure 4.

Pharmacokinetics Study

A pharmacokinetics study was carried out in male rats. After a single s.c. injection of either: 1) 100 mg/kg dose of leptin (20 mg/ml) formulated in 20mM acetate buffer, pH 4.8); or 2) a leptin/hydrogel (74/26% (PLGA/PEG)(w/w)) formulation consisting of 20 mg/ml leptin, in 20mM acetate, pH 4.8, blood samples were collected at various time intervals and analyzed for leptin by ELISA assay. As shown in Figure 5, serum concentrations of leptin were detectable for up to 168 hours for animals injected with the leptin/hydrogel formulation.

Example 5

This example describes the incorporation of G-CSF into the hydrogel and the results of *in vitro* release studies using the formulation.

GCSF solution (formulated in 10mM acetate, 5% sucrose, pH 4.0) was added to the copolymer hydrogel solution (formulated in 20mM acetate, pH 6.0) as described in Example 4. The final concentration of the copolymer in the GCSF/hydrogel formulation was 10-50% (w/w) and the GCSF concentration was in the range of 1-20 mg/ml. The *in vitro* release of GCSF from the hydrogel was carried out in 20mM sodium phosphate buffer, pH 7.4, at 37°C as described in Example 4.

The % GCSF released over time is shown in Figure 6. As depicted in Figure 6, nearly 100% of the GCSF is released over a 9-10 day period of time. The integrity of the GCSF released from the hydrogel formulation was confirmed by HPLC (data not shown) and gel electrophoresis (SDS-PAGE)(see Figure 7).

Example 6

This example describes the incorporation of an Fc-OPG protein into the hydrogel and the results of *in vitro* release studies using the Fc-OPG/hydrogel formulation.

The Fc-OPG/hydrogel formulation was prepared as described in Example 4 by adding Fc-OPG solution (formulated in 10mM sodium acetate, 5% sorbitol, 0.02 mg/ml tween 20, pH 5.0) to the copolymer solution (formulated in 50mM acetate, pH 6.0). The *in vitro* release of Fc-OPG from the hydrogel was carried out in 20mM sodium phosphate buffer, pH 7.4, at 37°C as described in Example 4. The % Fc-OPG released over time is shown in Figure 6. As depicted in Figure 6, nearly 100% of the Fc-OPG is released over a 8-9 day period of time.

Example 7

This example describes incorporation of the Zn:leptin suspension into PLGA/PEG hydrogel and the results of *in vivo* release kinetics of the leptin from the Zn:leptin/hydrogel.

The PLGA/PEG block polymers described in the examples above were hydrated in 100mM Tris, pH 8.0 buffer. The final pH of the hydrogel solution was maintained between 6.5 - 7.0 and then a zinc chloride

solution was added to the hydrogel to obtain a 0.1mM $ZnCl_2$ concentration in the final hydrogel solution. To this hydrogel solution, a Zn:leptin suspension was added as described in Example 4. The final Zn:leptin concentration in the hydrogel described in this example was 20 mg/ml. The *in vivo* bioactivity of a Zn:leptin/hydrogel (74/26% (PLGA/PEG)(w/w)) formulation was carried out as described in Example 4. The results of the *in vivo* bioactivity studies are depicted in Figure 8.

Example 8

This example describes the incorporation of Zn:GCSF into the PLGA/PEG hydrogel and the results of *in vitro* release studies using the formulation.

The PLGA/PEG block copolymer described in the examples above was hydrated in 100mM PIPES, pH 7.5 buffer. The final pH of the hydrogel solution was maintained between 6.5 - 7.0 and then a zinc chloride solution was added to the hydrogel to obtain a 0.1mM $ZnCl_2$ concentration in the final hydrogel solution. To this hydrogel solution, a Zn:GCSF suspension was added as described in Example 4. The *in vitro* release of GCSF from the hydrogel was carried out in 20mM sodium phosphate buffer, pH 7.4, at 37°C, as described in Example 4. It was demonstrated that sustained release of GCSF could be obtained from these hydrogel formulations.

Example 9

This example describes the incorporation of GCSF-crystals in the PLGA/PEG hydrogel and the results of *in vitro* release studies using the formulation.

The block polymer described in the examples above was hydrated in 100mM MES, pH 7.5 buffer. The final pH of the hydrogel solution was maintained between 6.5 - 7.0 and then a $MgCl_2$ solution was added to the hydrogel to obtain a 0.2M $MgCl_2$ concentration in the final hydrogel solution. To this hydrogel solution, a GCSF crystals suspension was added as described in Example 4. The *in vitro* release of GCSF from the hydrogel was carried out in 20mM sodium phosphate buffer, pH 7.4, at 37°C, as described in Example 4. It was demonstrated that sustained release of GCSF could be obtained from these hydrogel formulations.

Example 10

This example describes the effect of various excipients on the LCST of PLGA/PLGA, A-B-A block copolymers. As indicated in Table 4 below, the addition of various sugars, salts, surfactants, etc. can effect the rate of gelation and LCST of the hydrogels.

Table 4

Excipient Added	Effect on Gelation	Effect on LCST
Sugars at 5%-20% (e.g. glucose, sucrose)	↑ rate of gelation Firm gel	Lowered LCST
Salts at 0.5%-10% (e.g. $NaCl_2$, $ZnCl_2$, Na_2SO_4)	↑ rate of gelation Firm gel	Lowered LCST
Surfactants (e.g. Tween, SDS)	↓ rate of gelation Soft gel	-----
Glycerol at 2%-10% (e.g. $NaCl_2$, $ZnCl_2$, Na_2SO_4)	↑ rate of gelation Firm gel	Lowered LCST
Polyethylene glycol at 5%-20%	↓ rate of gelation Soft gel	Increased LCST

The present invention has been described in terms of particular embodiments found or proposed to comprise preferred modes for the practice of the

invention. It will be appreciated by those of ordinary skill in the art that, in light of the present disclosure, numerous modifications and changes can be made in the particular embodiments exemplified without departing from the intended scope of the invention.

WHAT IS CLAIMED IS:

1. A pharmaceutical composition for the sustained administration of an effective amount of a biologically active agent, or a derivative, analog, fusion, conjugate, or chemically modified form thereof, comprising an injectable biodegradable polymeric matrix into which said biologically active agent has been incorporated, said polymeric matrix having reverse thermal gelation properties, and wherein said injectable polymeric matrix is maintained at a temperature below the lower critical solution temperature of said polymeric matrix.

2. The composition of claim 1, wherein said polymeric matrix is a biodegradable block copolymer comprising:

(a) 55% to 90% by weight of a hydrophobic A polymer block and;

(b) 10% to 45% by weight of a hydrophilic B polymer block comprising a polyethylene glycol having an average molecular weight of between 500-10000.

3. The composition of claim 2, wherein said hydrophobic A polymer block is a poly(α-hydroxy acid) having an average molecular weight of between 1000-20,000.

4. The composition of claim 3, wherein said poly(α-hydroxy acid) is selected from the group consisting of poly(lactide)s (d,l- or l- forms), poly(glycolide)s, polyanhydrides, polyorthoesters, polyetheresters, polycaprolactone, polyesteramides,

polycarbonate, polycyanoacrylate, polyurethanes, polyacrylate, blends and copolymers thereof.

5. The composition of claim 4, wherein said poly(α-hydroxy acid) is poly lactide-co-glycolide (PLGA).

6. The composition of claim 5, wherein said block copolymer is a triblock copolymer having a configuration selected from the group consisting of ABA or BAB block segments.

7. The composition of claim 6, wherein said hydrophobic A polymer block comprises 74% by weight of said block copolymer and said hydrophilic B polymer block comprises 26% by weight of said block copolymer.

8. The composition of claim 7 further comprising an excipient which will vary the lower critical solution temperature and increase the rate of gelation of said block copolymer.

9. The composition of claim 1, wherein said biologically active agent is a protein selected from the group consisting of interferon consensus, interleukins, erythropoietins, granulocyte-colony stimulating factor (GCSF), stem cell factor (SCF), leptin (OB protein), interferons (alpha, beta, gamma), tumor necrosis factor (TNF), tumor necrosis factor-binding protein (TNF-bp), interleukin-1 receptor antagonist (IL-1ra), brain derived neurotrophic factor (BDNF), glial derived neurotrophic factor (GDNF), neurotrophic factor 3 (NT3), fibroblast growth factors (FGF), neurotrophic growth factor (NGF), bone growth factors such as osteoprotegerin (OPG), granulocyte

macrophage colony stimulating factor (GM-CSF), megakaryocyte derived growth factor (MGDF), keratinocyte growth factor (KGF), thrombopoietin, platelet-derived growth factor (PGDF), tissue plasminogen activator (TPA), urokinase, streptokinase and kallikrein.

10. The composition of claim 1, wherein said biologically active agent is a small molecule.

11. A method for the parenteral administration of a biologically active agent, or a derivative, analog, fusion, conjugate, or chemically modified form thereof, in a biodegradable polymeric matrix to a warm blooded animal with the resultant sustained release of said agent concomitant with biodegradation of said polymeric matrix, which comprises:

(a) providing an injectable liquid polymeric matrix comprising a biodegradable block copolymer having reverse thermal gelation properties, and into which a biologically active agent has been incorporated;

(b) maintaining said liquid polymeric matrix at a temperature below the lower critical solution temperature of said polymeric matrix; and

(c) injecting said liquid parenterally into said animal, thus forming a gel depot of said drug and polymeric matrix as the temperature of said liquid is raised in the body of said animal above the lower critical solution temperature of the polymeric matrix.

12. The method of claim 11, wherein said polymeric matrix is a biodegradable block copolymer comprising:

(a) 55% to 90% by weight of a hydrophobic A polymer block and;

(b) 10% to 45% by weight of a hydrophilic B polymer block comprising a polyethylene glycol having an average molecular weight of between 500-10000.

13. The method of claim 12, wherein said hydrophobic A polymer block is a poly(α-hydroxy acid) having an average molecular weight of between 1000-20,000.

14. The method of claim 13, wherein said poly(α-hydroxy acid) is poly lactide-co-glycolide (PLGA).

15. The method of claim 14, wherein said block copolymer is a triblock copolymer having a configuration selected from the group consisting of ABA or BAB block segments.

16. The method of claim 15, wherein said hydrophobic A polymer block comprises 74% by weight of said block copolymer and said hydrophilic B polymer block comprises 26% by weight of said block copolymer.

17. The method of claim 16 further comprising an excipient which will vary the lower critical solution temperature and increase the rate of gelation of said block copolymer.

18. The method of claim 11, wherein said biologically active agent is a protein selected from the group consisting of interferon consensus, interleukins, erythropoietins, granulocyte-colony

stimulating factor (GCSF), stem cell factor (SCF), leptin (OB protein), interferons (alpha, beta, gamma), tumor necrosis factor (TNF), tumor necrosis factor-binding protein (TNF-bp), interleukin-1 receptor antagonist (IL-1ra), brain derived neurotrophic factor (BDNF), glial derived neurotrophic factor (GDNF), neurotrophic factor 3 (NT3), fibroblast growth factors (FGF), neurotrophic growth factor (NGF), bone growth factors such as osteoprotegerin (OPG), granulocyte macrophage colony stimulating factor (GM-CSF), megakaryocyte derived growth factor (MGDF), keratinocyte growth factor (KGF), thrombopoietin, platelet-derived growth factor (PGDF), tissue plasminogen activator (TPA), urokinase, streptokinase and kallikrein.

19. The method of claim 11, wherein said biologically active agent is a small molecule.

FIG. 1

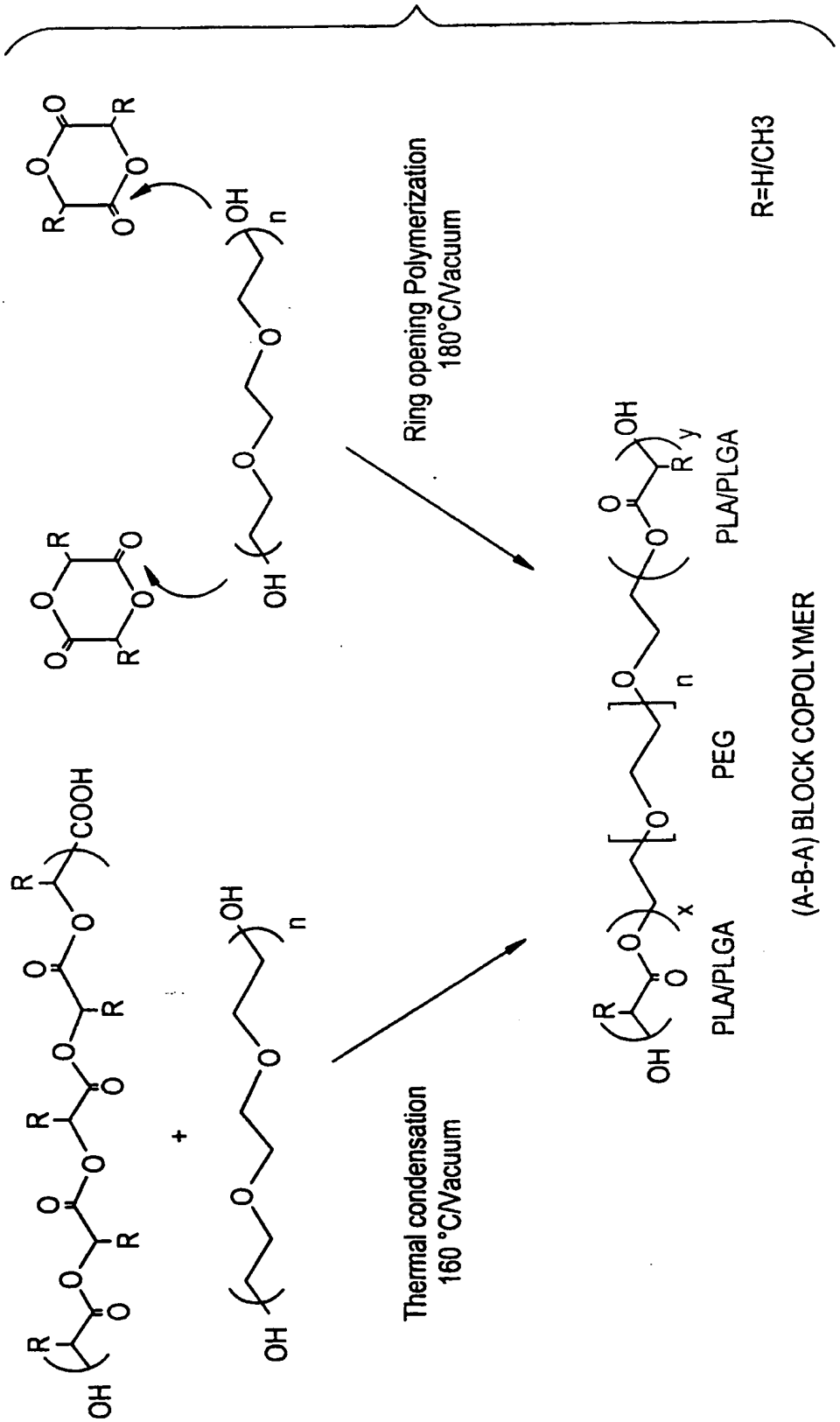

Ring opening Polymerization
180°C/Vacuum
Thermal condensation
160 °C/Vacuum
R=H/CH3
PLA/PLGA
PEG
PLA/PLGA
(A-B-A) BLOCK COPOLYMER

FIG. 2

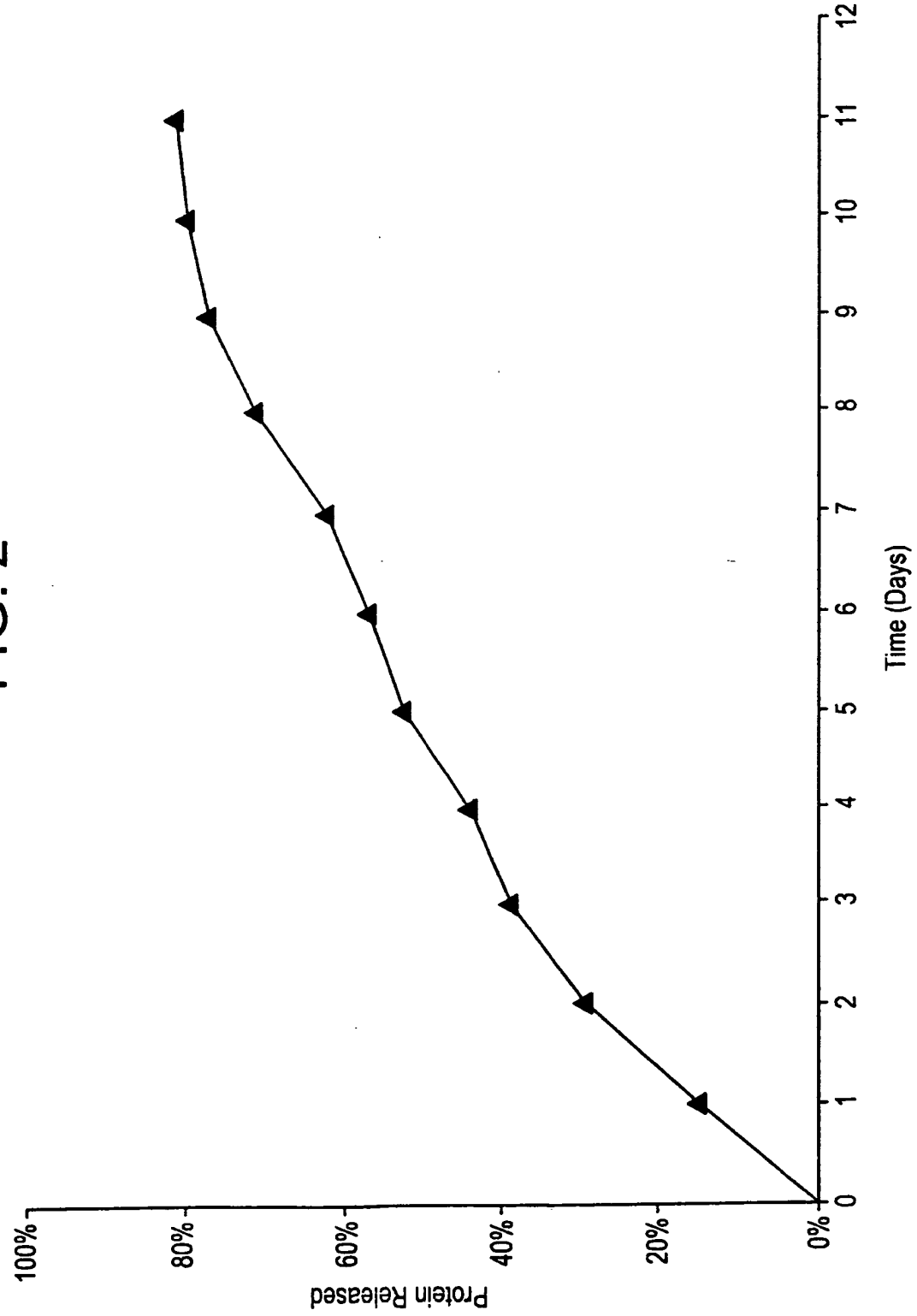

Protein Released
100%
80%
60%
40%
20%
0%
0
1
2
3
4
5
6
7
8
9
10
11
12
Time (Days)

FIG. 3

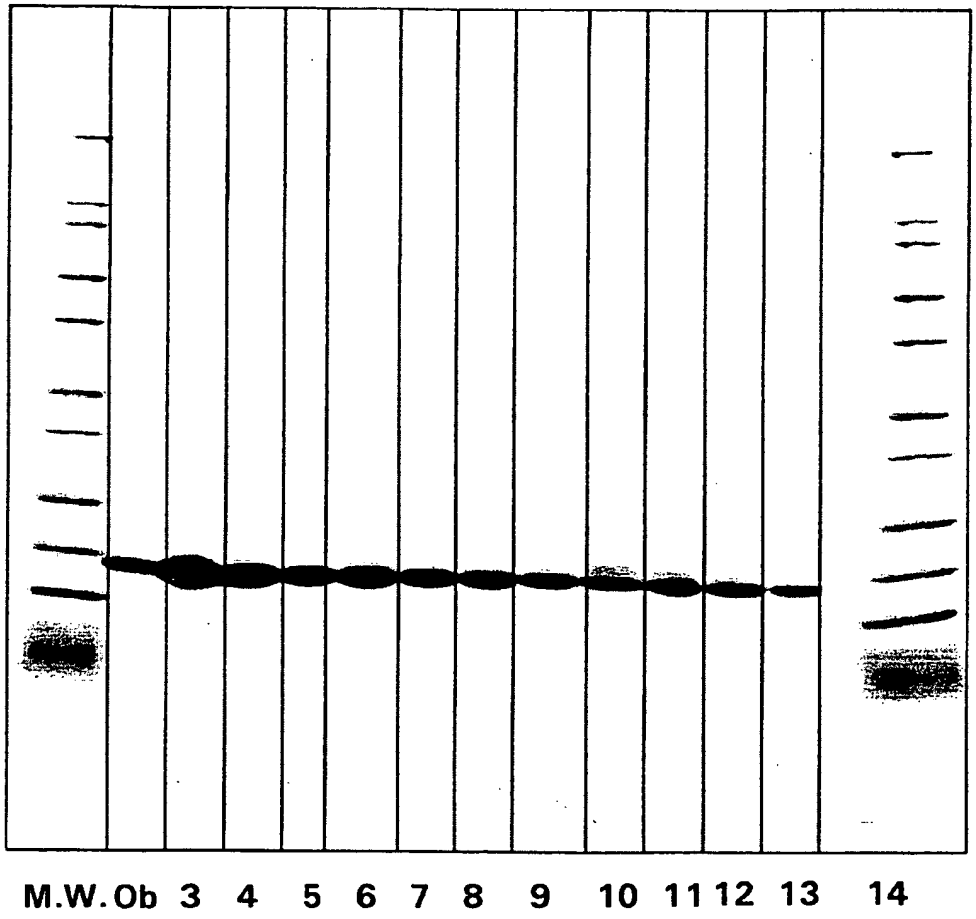


FIG. 4

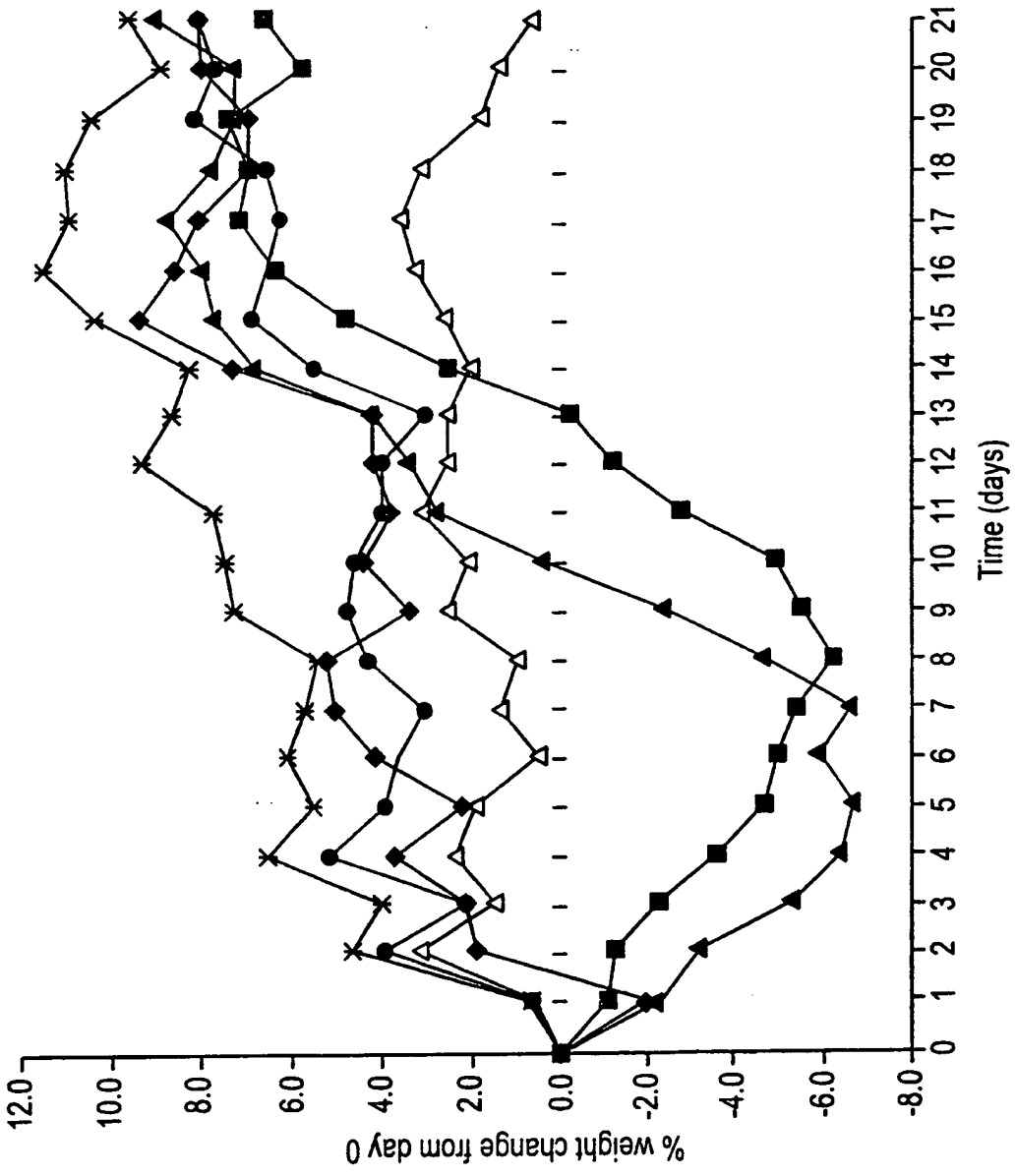

% weight change from day 0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
-2.0
-4.0
-6.0
-8.0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Time (days)

FIG. 5

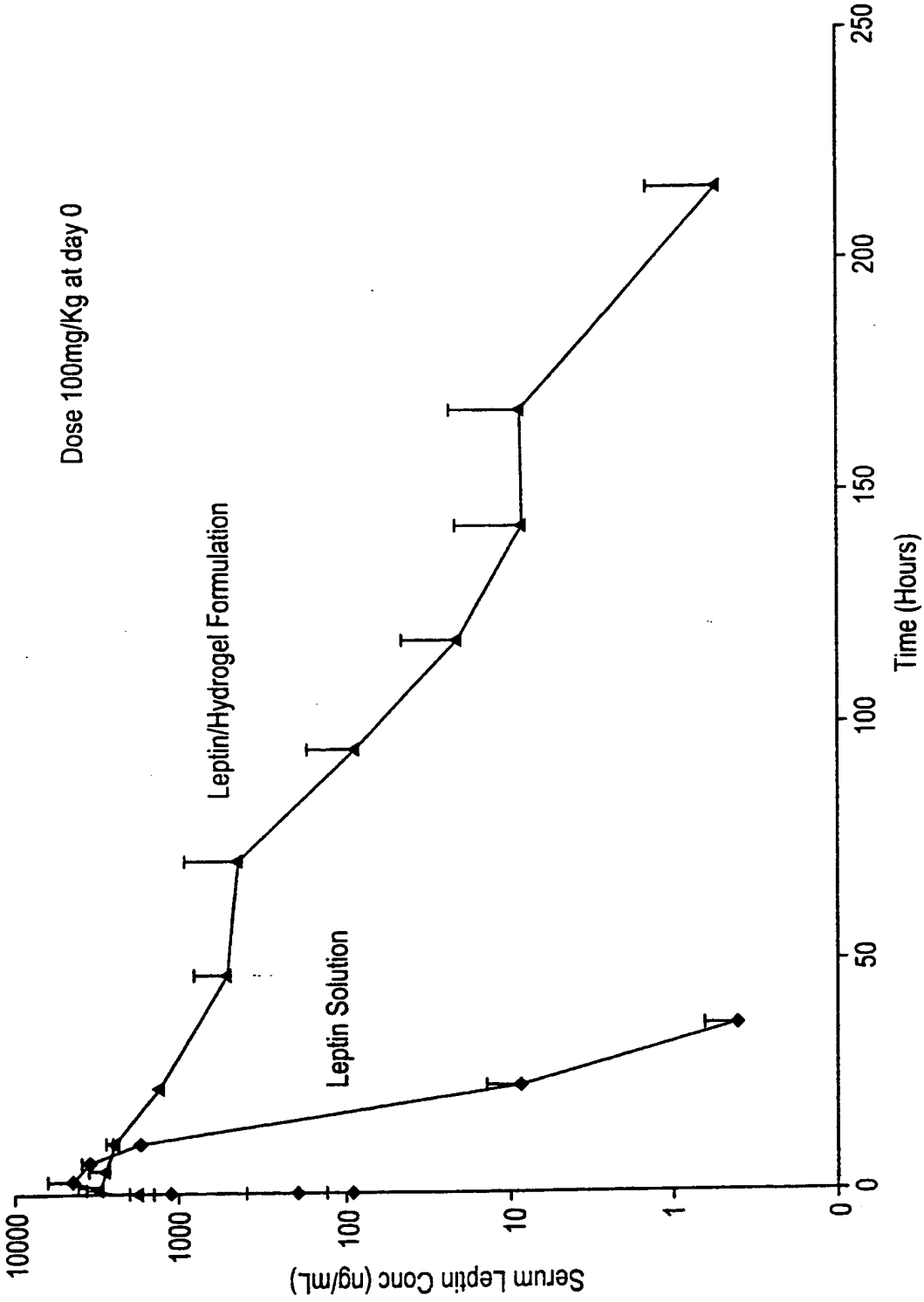

Dose 100mg/Kg at day 0
Leptin/Hydrogel Formulation
Leptin Solution
Serum Leptin Conc (ng/mL)
10000
1000
100
10
1
0
0
50
100
150
200
250
Time (Hours)

FIG. 6

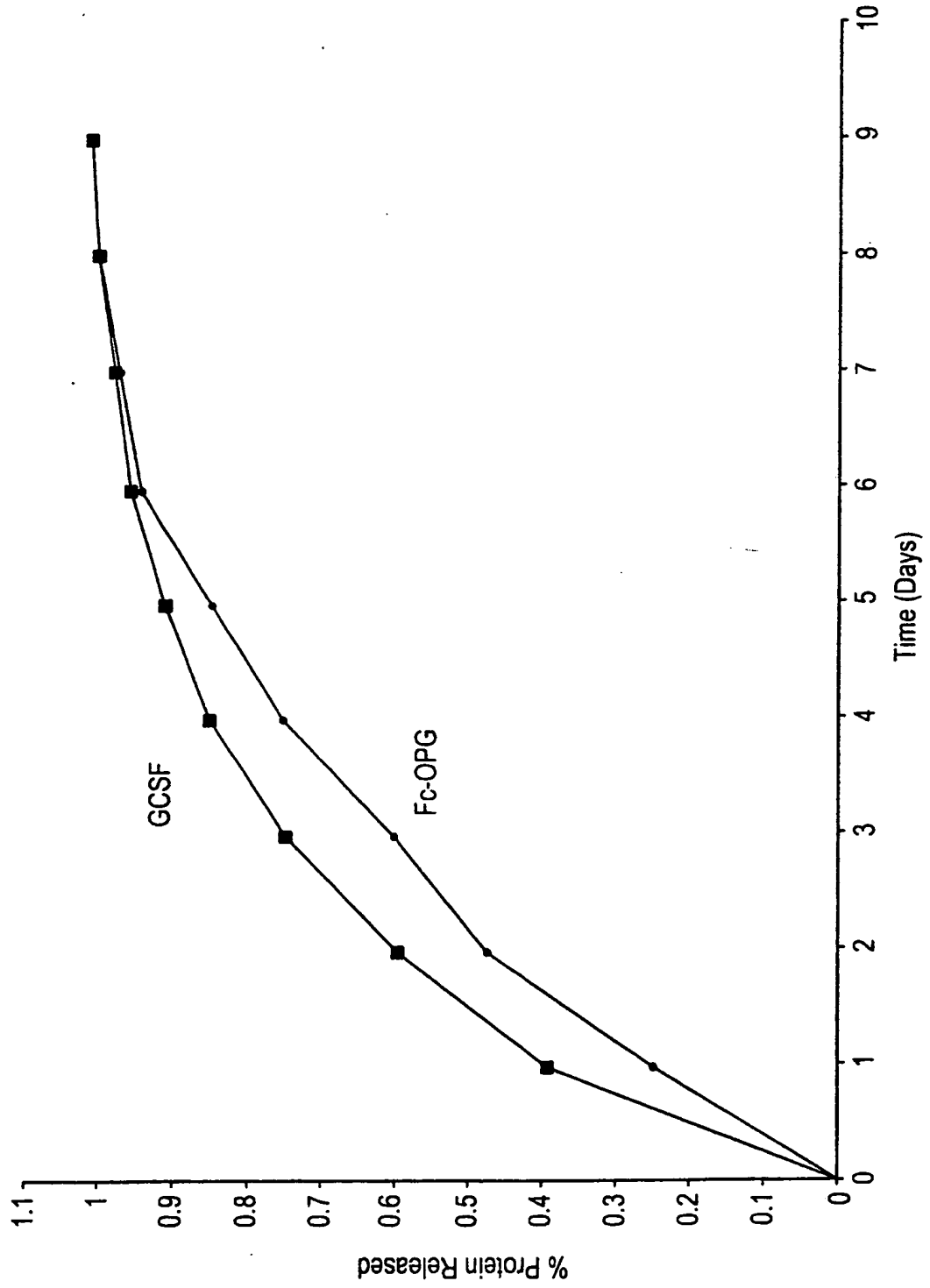
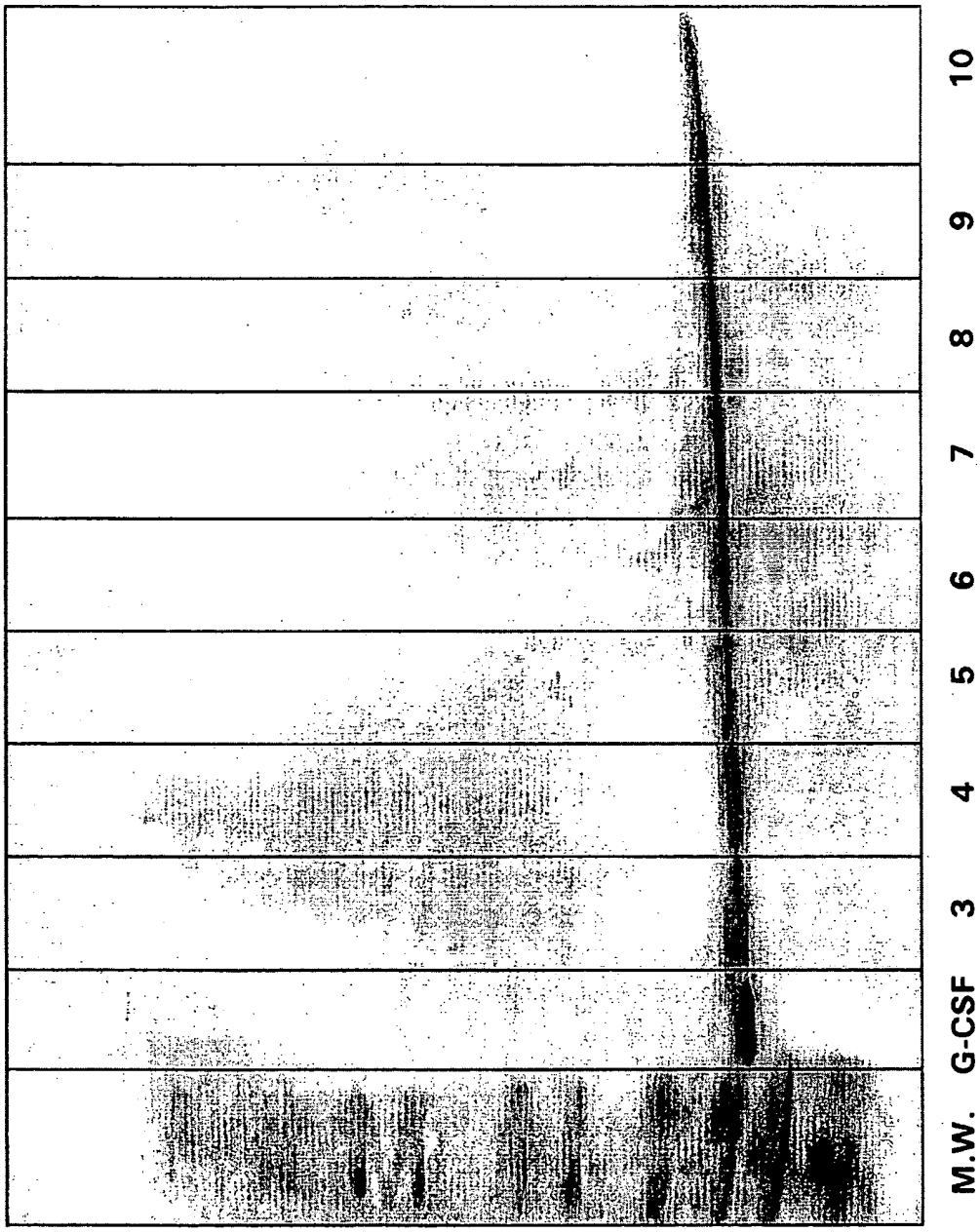

% Protein Released
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Time (Days)
0
1
2
3
4
5
6
7
8
9
10
GCSF
Fc-OPG

FIG. 7



INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/14206

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K47/34

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 99 18142 A (MACROMED, INC.) 15 April 1999 (1999-04-15) the whole document ---	1-19
X	WO 97 15287 A (MACROMED, INC.) 1 May 1997 (1997-05-01) & US 5 702 717 A cited in the application -----	1,9-11, 18,19

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 November 1999	15/11/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Benz, K

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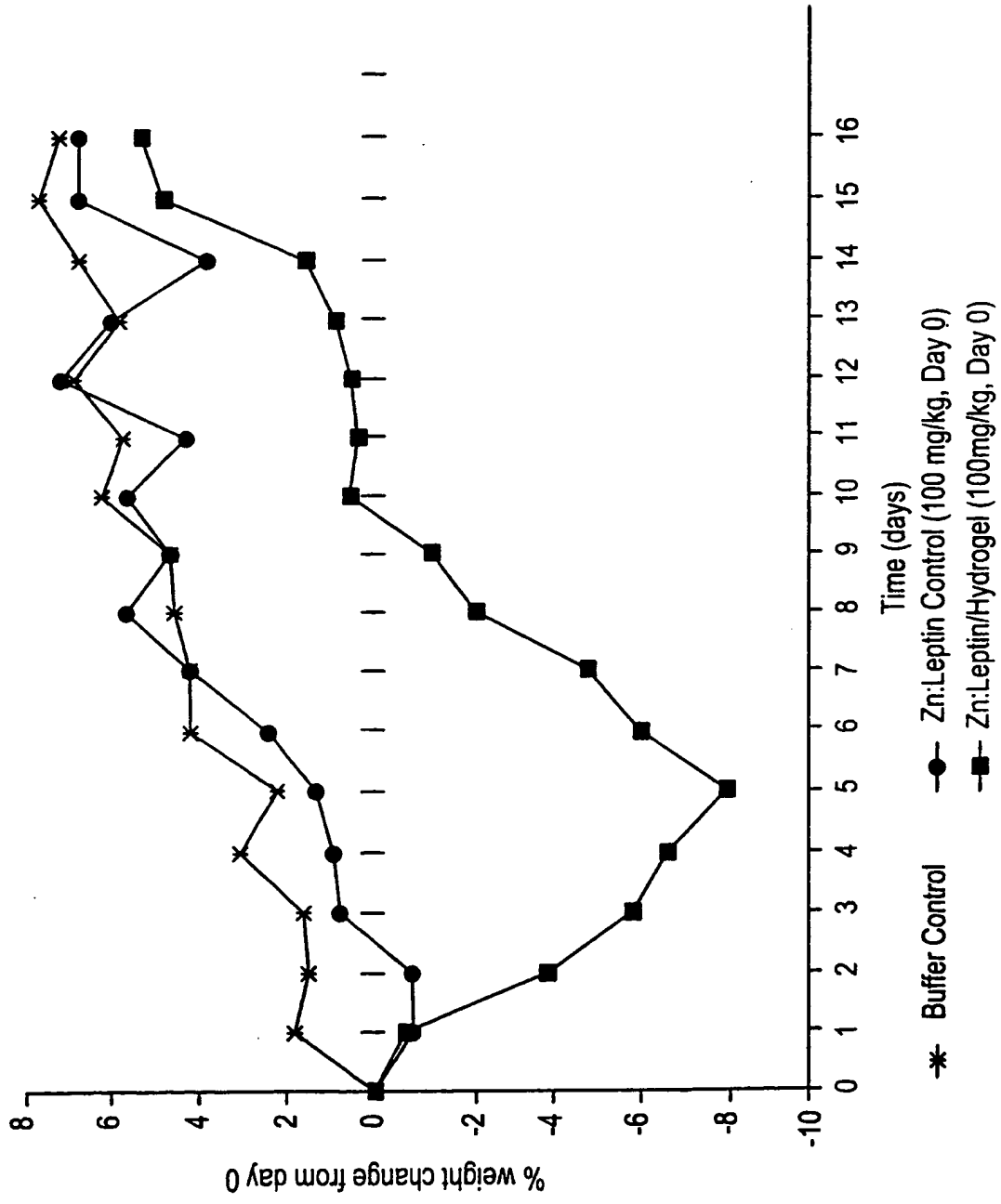
FIG. 8


% weight change from day 0
8
6
4
2
0
-2
-4
-6
-8
-10
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Time (days)
Buffer Control
Zn:Leptin Control (100 mg/kg, Day 0)
Zn:Leptin/Hydrogel (100mg/kg, Day 0)

INTERNATIONAL SEARCH REPORT

ernational application No.
PCT/US 99/14206

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 11-19 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

nformation on patent family members

Inte··ational Application No
PC·/US 99/14206

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9918142 A	15-04-1999	AU 9678098 A	27-04-1999
WO 9715287 A	01-05-1997	US 5702717 A	30-12-1997
		AU 7520096 A	15-05-1997
		EP 0863745 A	16-09-1998